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Editor-in-Chief, MARTIN FROBISHER
374 Ruckman Road
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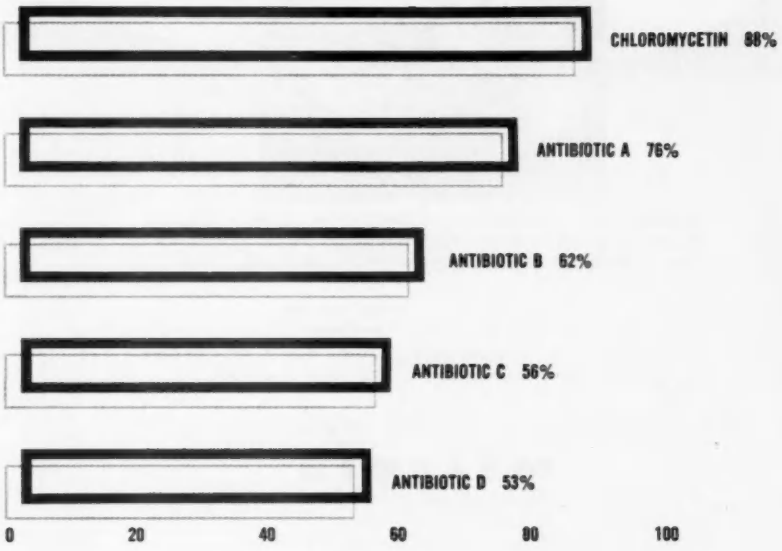

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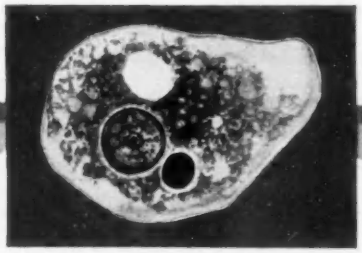

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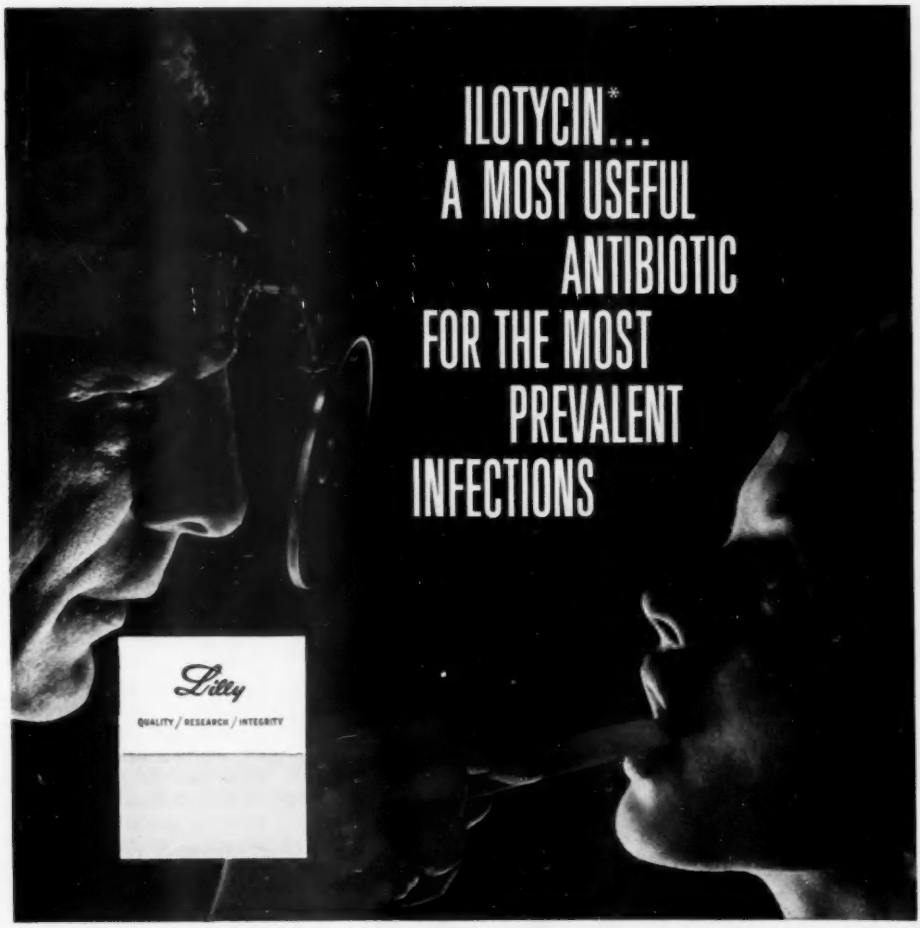

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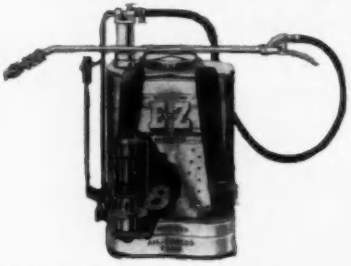

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TROPICAL ALLERGENS AND ALLERGIC DISEASES

Presidential Address before the 1957 Annual Meeting of the American Society of Tropical Medicine and Hygiene

JUSTIN M. ANDREWS

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 14, Maryland

Prominent among the presidential duties specified in the By-Laws of this Society is the delivery of an address at its annual meeting. Fortunately, at least in my own opinion, no restrictions or indications as to its subject matter have been laid down. Consequently, the presidential addresses made during the period of my association with this Society (now more than 30 years) reflect the diverse interests, concerns and drives of my distinguished predecessors in this high office. Some of them have dealt with purely technical considerations of one or more aspects of tropical disease or of basic or applied research in related fields in which they were masters. Others have stressed the importance of tropical medicine and hygiene in world health and economy, or have summarized its accomplishments in the past, or have even ventured to prognosticate its future. Still others have philosophized significantly about its relation to world food supplies, to global warfare, to the postwar world, to increasing population pressures, and to other events or circumstances of current, cosmopolitan, or humanitarian importance.

In this connection, I was much impressed by Dr. Chandler's (1957) masterly discourse last year on the interrelations of malnutrition and infectious disease in the tropics because it had the salutary effect of enlarging our concept of tropical disease. I have long been disturbed, as I (1957) indicated to you earlier this year in the pages of the "Tropical Medicine and Hygiene News," by the unbecoming truth that most of us in this country who profess to be interested in tropical medicine and hygiene have decidedly tubular vision with reference to the total spectrum of disease in the tropics. This, I am sure, is due primarily to the fact that the majority of the members of this Society are laboratory and research oriented. By training and experience we are, for the most part, microbiologists of one subspecies or another. Our knowledge, competences, and our interests, therefore, pertain mainly to the infectious and parasitic maladies of warm areas. The clinical and administrative moiety of our membership also may have greater interest in these same categories of illness because they are the more importunate diseases of the tropics, occurring frequently, exhibiting spectacular syndromes, being readily diagnosable by laboratory as well as clinical study, and usually yielding readily to standardized chemotherapeutic regimes.

I have no desire to belittle or deprecate the infectious and parasitic diseases (indeed, I would be defiling my own nest if I did) but I am sure that these are not the totality of tropical disease, and if we are to identify ourselves as students and authorities in this area, we should be aware of, and know something about, the noninfectious and nonparasitic morbidity which is peculiarly associated with the tropics. To this end, Dr. Chandler (1957) has directed our attention to the large and important group of nutritional diseases. I propose to do something of the sort for another category of health impairments about which we hear little in this Society, viz., the allergic diseases. That I should have the temerity to discuss such a vast and complex subject so far removed from my own field or professional indoctrination will come, I am sure, as a severe shock to most of my audience. I can only assure you that I harbor no illusions of grandeur with respect to my meager knowledge of allergy and allergic diseases. It happens, however, in my new ecologic niche, that I find myself with able and gracious colleagues[1] whose identification and attainments in the field of allergy are probably unsurpassed. Without implying in any way that they should share with me accountability for the validity of the argument propounded below, I gratefully acknowledge their patient and generous counsel in its preparation.

[1] I am referring to Dr. Jules Freund, Laboratory of Immunology, and to his staff in the National Institute of Allergy and Infectious Diseases.

Having these splendid resources at hand, however, was not my principle reason for selecting this subject. Numerous surveys and statistics indicate that allergies are important primary and complicating causes of human discomfort and disease in this country. Some 10 per cent of our population, nearly 17 million people, are reckoned to have frank or major impairments of health due to sensitivities of various sorts, and an additional 40 to 50 per cent are said to have experienced or will experience some transient or minor allergic manifestation (Vaughan and Black, 1954). Deaths, usually due to uncontrolled anaphylactic shock, also occur but their number is relatively insignificant (Vaughan and Pipes, 1936).

It seemed to me improbable that tropical residents would be so constitutionally different that such a highly prevalent group of disorders would be restricted to temperate-zone residents, and I began to wonder whether or not the situation was at all comparable in the tropics. I had spent some time in the tropical areas of both hemispheres but I could not recall hearing much, if anything, about diseases such as hay fever or asthma, considered to be solely or predominantly allergic in their etiology and pathogenesis. Nor could I remember that such subjects had been discussed in Society meetings or in our Journal, with the notable exception, of course, of those parasitic or infectious tropical diseases such as filariasis, malaria, leprosy, ascariasis, and many more in which allergic reactions are present but are clearly accessory to other reactions of more primary etiologic significance. I scanned the subject indices and tables of contents of the "American Journal of Tropical Medicine and Hygiene" for the last 10 years and found, for whatever it means, that the word "allergic" and its congeners used in reference to strictly primary etiology appeared only a couple of times. In the Second, Third, Fourth, Fifth, and in the proposed Sixth International Congresses of Tropical Medicine and Malaria there was, or is, no section devoted to allergic diseases, and in the Proceedings of the past Congresses I could find only one article in this field (Ambler, 1948). The same is true of the Far Eastern Association of Tropical Medicine and its Transactions in which I located another single contribution on allergic disease (Toms, 1930). On the other hand, it was easily ascertained that the allergist and his special interests are well represented in the medical literature and conferences of the temperate zones. I concluded that allergic disease either was not as important in the tropics as I had surmised or that it was not being recognized and brought to the attention of persons in this country (and in others) most identified with knowledge of tropical medicine and hygiene. My curiosity as to which of these possibilities was correct led me to consultation and library research on this subject. The latter involved scanning much of the pertinent literature of tropical medicine, parasitology, and allergy, which turned out to be far more voluminous than I had anticipated when I so casually undertook this task. Accordingly, I have been obliged to deal with the subject more restrictively and superficially than its importance deserves.

It is not feasible to cover at this time anything like the entire spectrum of disease-related allergic manifestations in the tropics, and so I must delineate the particular segment which I propose to discuss. The word "allergy" was suggested in 1906 by von Pirquet, an Austrian pediatrician, who took exception to the term "anaphylaxis," used previously by Charles Richet (Portier and Richet, 1902) as the antonym of "prophylaxis," to describe what Richet and his collaborators regarded as a loss of natural immunity against certain substances when these were reinjected into animals after several days. The word "allergy" was more acceptable than "anaphylaxis" to many workers in this new science because it indicated only an altered capacity to react without implying how or why it came about. Furthermore, it was a much broader term which could be applied as appropriately to diminished as to increased capacities to react. Thus, as emphasized by Opie (1936), allergic manifestations include sensitization, i.e., abnormally increased response to insult, and/or immunization, characterized by antibody formation and enhanced resistance to infection. Curiously enough, these two apparently antithetical states are not mutually exclusive and may actually coexist or succeed each other in the same body. In this presentation, however, I am deliberately avoiding involvement in the immunogenic and immunodiagnostic aspects of tropical disease as these special components of the broad subject of allergenesis have been treated in textbooks and original articles known to many of you. Rather, I propose to deal primarily with the tropical morbidity caused or enhanced by allergens, i.e., specific substances to which certain

individuals exhibit unusual and exaggerated sensitivities but which are harmless in similar amounts to the vast majority of the population (Dorland, 1951).

Reactions to allergens may occur when these substances touch the skin or mucosa, when they are ingested or inhaled or when they are introduced otherwise into the body. They may produce such well-known symptom complexes as urticaria, dermatitis, and ulceration of the skin and mucosa; violent gastrointestinal disturbances; various forms of rhinitis and associated symptoms; acute respiratory distress, including asthma; and a host of other disorders among which migraine and ulcerative colitis are commonly included. In this connection, it is important to discriminate in our thinking between the allergens, as defined above, and other substances known as primary toxicants or irritants which, in relatively minute quantities, regularly produce in virtually all exposed persons irritation, inflammation, and a variety of other increasingly severe toxic and pathologic developments. The sap of the manchineel or beach-apple tree, *Hippomane mancinella*, common in the West Indies, is a well-known example of a primary toxicant. Certain substances, like the venom of stinging insects, may possess both allergic and primary irritant qualities (Benson and Semenov, 1930; Jex-Blake, 1942), and this is also true of some plant materials. The term "allergy" has been applied somewhat indiscriminately to both types of reaction, and it is possible that future studies may reveal that their differences are more quantitative than qualitative. In this presentation, however, I am trying to exclude from consideration what appear to be primary toxicants, though this is not always easy to do on the basis of information available.

I commenced this literary inquiry by consulting virtually all of the well-known textbooks of tropical medicine. I could not read through all of them and so I searched tables of contents and subject indices, and read selected portions of the text where it seemed probable that allergy and hypersensitivity might be mentioned. I discovered that as long ago as 1905, in the first edition of Mense's "Handbuch der Tropenkrankeiten," Van Brero (1905) of Java had pointed out in a section of the first volume devoted to nervous and psychic disturbances that "rhinitis spastica vasomotoria," first described, according to him, in 1901 by an otolaryngologist in Batavia named Zegers (1901), bore a strong clinical resemblance to hay fever, a word in general use by that time, and that this disease was probably caused by dusts acting upon the nasal mucosae of persons whose nervous systems had lost much of their natural resistance due to long residence in the tropics.

This assumption appears to have been dutifully copied into subsequent texts on tropical medicine, but for the most part the purely allergic diseases were given a wide berth by most textbook writers until recent years. Even now, much of the consideration of allergy in modern works on tropical diseases consists of references to infections or parasitisms complicated by sensitivity reactions, to flora which may be poisonous or allergic, to the significance of high eosinophilia, to the phenomenal hypersensitivity possessed by some to *Ascaris*, and to the untoward reactions of occasional individuals to drugs used in the treatment of tropical diseases. With the exception of the late editions of Manson-Bahr (1951) and Strong's Stitt (1944), little or nothing is said about the specific allergens of the tropics which may cause rhinitis, asthma, migraine, or enteric disturbances. Perhaps this is because most tropical-disease textbook writers, like most members of the American Society of Tropical Medicine and Hygiene, are not allergists! I may add, parenthetically, that, based upon a simliar perusal of the principal textbooks on allergy, I found little or no mention of the role of allergy in complicating tropical infectious and parasitic diseases.

I turned next to nosogeographic sources of information and reviewed Earl Baldwin McKinley's (1935) monumental "Geography of Disease," modestly subtitled "A Preliminary Survey of Tropical and Certain Other Diseases." This was published in 1935 as a Supplement to the Journal of this Society and was financed by The American Leprosy Foundation. It is a 495-page volume reporting the findings of a National Research Council Advisory Committee on Survey of Tropical Diseases.[2] It consists largely of tabular arrays of responses to a carefully designed questionnaire sent to tropical and subtropical health authorities

[2] The members of this Committee were Dr. Frederick P. Gay, Chairman; Dr. Richard P. Strong, and Dr. Theobald Smith. Dr. McKinley was the Director of Studies. Dr. Smith died before the report was published, and it is dedicated to his memory.

throughout the world. The questionnaire provided space for reports concerning the occurrence and health importance of some 80 "Tropical Diseases" and of 32 other "Diseases Usually Considered as of Temperate Climates." All of the disorders named were of infectious, parasitic, metabolic, nutritional, degenerative, or unknown origin. While allergic manifestations undoubtedly contributed to the total pathology of many, it is unlikely that any one of them would be labelled an allergic disease in the sense that it was caused primarily by allergens. Thus, no information concerning the occurrence, distribution, or public health importance of allergic diseases was obtained from this study. I have mentioned it to highlight the fact that in the early '30's, public health leaders, not only in tropical but in temperate-zone areas, were thinking more in terms of infectious and communicable than of allergic etiology, though by that time most of the specific clinical allergies had been well characterized.

Shortly thereafter, however, scientific notes and articles by allergists, who seem to be facile and prolific writers, became more numerous. Many of these related to the occurrence of pollens, fungal spores, special foods, infectious materials, drugs, animal "epidermals," and other sensitizing substances in particular places and regions of the world, including the tropics. In due course, these observations were gathered together in the textbooks on allergy and in monographs concerning special manifestations of allergic states such as hay fever and asthma. Many of these were summarized in a three-volume production familiar to most of you and inspired by one of our past presidents, Brigadier General James Stevens Simmons (1944, 1951, 1954). This was entitled "Global Epidemiology. A Geography of Disease and Sanitation." It is a narrative assembly of data regarding medical, health, and sanitary conditions of various geographic areas of the world, and is based mainly on surveys made for the Preventive Medicine Service of the United States Army, though information from other sources was also incorporated. The publication of these three volumes spans a 10-year period, 1944 to 1954; thus, the contents of the later volumes are based on more recent information than are the earlier ones. The account for each country or island location includes a brief paragraph on "Miscellaneous Conditions" in which the extremely scanty information then extant about allergens and allergic diseases is recorded. From these precis, supplemented by other published sources of information, it is possible to develop a fragmentary impression of the allergic risks in the tropics, area by area, though quantitative data regarding frequency of occurrence are scanty. Much more information is available for the American than for the Asian-Pacific or the African tropics.

According to Castellani, writing in 1938, hay fever and asthma were not at all rare in the tropics though not much was known about them. He believed that they occurred in all tropical and subtropical areas and had observed them in Ceylon, tropical Africa, and Central America. He was convinced that tropical hay fever was clinically identical with hay fever in temperate zones and that only the etiologic allergens differed. He stated that in Africa, hay fever was often associated with euphorbiaceous plants (*Euphorbia candelabrum* and relatives), some of the erect veldt grasses, especially *Egrarostris plana*, and some creeping grasses such as Bermuda grass (*Cynodon dactylon*). He referred to copra dust and tea dust as common allergens in Ceylon, but it has since been shown that the allergic manifestations associated with these substances, more commonly dermatitis than hay fever, are due to minute tyroglyphid mites (Mackie, Hunter, and Worth, 1954). He also mentioned sugar cane in relation to hay fever and the allergenic potentialities of ascarids and insects, living and dead, in the tropics.

The Dutch colonial medical officers in the Netherlands East Indies associated spastic rhinitis with pollens from bamboo, corn, sorghum, rice, coffee, tobacco (ter Heege, 1941), the oil palm (Tellings, 1941), and certain other palm species, except the royal palm. It was shown that persons with hay fever acquired either in Holland or the colonies were frequently skin-sensitive to extracts made from pollen originating from either tropical or temperate zone plants (Benjamins, 1932).

Stephens and Jordoni (1938) report that jute, the plant fiber from which burlap bags are made, is a cause of rhinitis and asthma in India. Unger (1945) believes that bronchial asthma must be very common in that country as its medical journals contain so many articles on this subject. In Ceylon, the products of the cinnamon tree, *Cinnamomum zeylanicum*, are said to have

allergic qualities as do the bacterial- or fungal-infected fibers produced on the kapok tree, *Ceiba pentandra* or *Eriodendron anfranctuosum*. Asthma is a frequent occurrence, judging by the admissions to government hospitals (Global Epidemiology, Vol. 1).

In this connection, it is interesting to note that Freeman, Oxon and Hughes (1938) in England, using Ceylonese bamboo pollen, together with the pollen of Indian maize and Jamaican sugar cane, demonstrated their close antigenic similarity to the pollens of English timothy grass. Of 123 timothy-positive hay fever cases, none of whom had lived where sugar cane, maize, or bamboo was grown, all but three were markedly skin-positive and 108 were very sensitive to sugar-cane pollen extract. Maize and bamboo skin reactions could not be distinguished from the positive timothy tests. Most cases successfully desensitized to timothy-grass pollen became nonsensitive to sugar-cane pollens, though not all in the same period of time. Lastly, it was shown that in terms of skin sensitivity the pollens of the exotic plants were more closely related to those from timothy grass than to those from native English garden flowers such as Michaelmas daisy, heather, and daffodil.

There is not much information available about plant or other allergens in tropical Africa. In Egypt and the Egyptian Sudan numerous grasses exist the pollens of which are suspected of being potential allergens, but the differentiation of these grass species is incomplete and their actual importance uncertain. The same is true in French West Africa. In Kenya, it is evident that "star-grass," known in the United States as Bermuda grass (*Cynodon dactylon*), and the golden wattle, a species of acacia (*Acacia podalyriifolia*), are frequently responsible for hay fever (Global Epidemiology, Vol. 2). The flowers of the pyrethrum-producing *Chrysanthemum cinerariae folium* cause contact dermatitis in certain invididuals (Tonking, 1936) concerned in making insecticidal extracts.

Hay fever, asthma, and eczema in Israel are caused by the pollens of Bermuda grass (Gutman, 1941, 1950) and to a lesser extent by those of the olive tree, *Olea europa* (Kessler, 1953). Nothing is known about allergenic pollen production in the rest of the Near East as far as I can ascertain.

For the American tropics, the botanical agents of respiratory allergy have been admirably reviewed by the Naranjos of Ecuador (1950, 1957), who report that the more important pollens are those of the weed genera *Amaranthus*, *Chenopodium*, and *Helianthus*, and the grasses known to us as Bermuda, velvet, rye, and Kentucky blue grass. These authors have made comparative studies of pollen types and concentrations, which are relatively low, in the atmospheres of Quito in Ecuador and of Cali in Colombia. Despite their differences in elevation, these municipalities have similar pollination calendars, dependent primarily on precipitation rather than temperature. The rains commence in September or October and continue until May. Early in this period, grass pollens increase in number. Cali has more tree pollens than Quito and each city has a predominance of its own amaranthine species, *A. quitensis* in Quito and *A. spinulosus* in Cali.

Similar studies have been made in Cuba by de la Riva (1951), who finds that grass, weed, and tree pollens are antigenically important and, surprisingly enough, that the pollens of sugar cane, the principal crop of the island, is of no consequence as far as inhalant and respiratory allergies are concerned. Cases of contact dermatitis, due to handling stems and leaves which contain a sensitizing oleoresin, are common among sugar-cane field workers. Tobacco dust is a frequent and potent allergenic substance to those concerned in tobacco processing in Cuba where this is one of the main industries.

As a matter of fact, in spite of the lush vegetation of the tropics, allergic morbidity due to pollens is probably less prevalent there than in temperate-zone areas. There are several reasons for this. In the first place, the troublesome kinds of ragweed which are such powerful sensitizers in this country, are essentially nontropical plants, due to their ecologic requirements. According to Allard (1943), who has studied the worldwide distribution of the genus *Ambrosia*, the more mischievous species are absent or of extremely rare occurrence between 20° North and South latitudes. In many parts of the world, they do not thrive even in the subtropical areas extending farther north and south from the equator. Secondly, as Naranjo points out, in the temperate zone countries with four seasons in the year, the periods of pollination for each botanical species are more clearly defined and the concentrations of specific pollen grains are usually higher. In tropical countries there are only two seasons,

in many areas hardly distinguishable, which may occur at different times in different places. Where plant flowering depends primarily on the rains, pollination periods are relatively clearcut but even under these conditions, the concentrations of pollens are never as high nor their anemophilous dissemination as distant as in temperate zones, due largely to the air-cleansing effect of the rains.

Nothing has been said thus far of other groups of sensitizing agents of special significance in the tropics. These include the air-borne spores of fungi, discovered in 1924 by van Leeuwen (1924) in Holland to be relatively common excitants of allergic reactions. Their importance in temperate climates was speedily confirmed by several observers and their occurrence and relationship to disease recorded. About a dozen genera are commonly involved though many more have been described. They have a broad, overlapping distribution in both temperate and tropical zones. Certain of these are important, however, in the tropics because they grow there more rapidly, especially in the rainy season, as I expect many of you can testify.

The first reference to them as causes of tropical allergies is by Ellis who, in 1933, noted the frequent occurrence of hay fever and asthma at Port Sudan on the Red Sea. He related this to the presence, during the damp months, of mold spores of undetermined types. Since then these spores have been studied, especially in the American tropics where, according to Naranjo, de la Riva, and Ribeiro (1937) of Brazil, the more important genera are *Alternaria*, *Aspergillus*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, and *Rhodotorula*. These vary in prevalence in different areas and at different seasons, and other genera are encountered at times. There seems to be general agreement, however, that in the American tropics at least, the fungal spores are much more important allergens than are the pollens (Naranjo, de la Riva, Ribeiro, and Quintero, 1955).

Another material which appears to be an important and common cause of rhinitis and asthma in the tropics of this hemisphere as well as in the temperate zones is that disreputable conglomerate known as house dust. Since Cooke's demonstration in 1922 that it contained substances which would react sharply with antibodies in the skin of asthmatics, house dust has been abundantly proved an important source of sensitization. Skin tests with house dust extracts variously controlled to reduce misleading results were positive in 35 to 79 per cent of persons with chronic or perennial asthma (Rimington, 1951). One might suppose that house dust would be of less significance in tropical situations where houses are open and well ventilated and where minimal use is made of cloth rugs and upholstered furniture. Nevertheless, house dust accumulates even under these conditions and continues to puzzle allergists who feel, in spite of its heterogeneous composition, that it contains unique allergenic substances which are more than, or different from, the sum of its identifiable allergenic constituents. Nor does it seem to make much difference where it comes from, as far as its sensitizing qualities are concerned. Southerland of Australia (1945), who has made intensive studies on this subject, concludes, "Whatever its origin, the allergen of house dust is found widely distributed throughout the world, and although some localities appear to yield more potent dust than others, qualitative studies carried out by the writer indicate that asthmatic patients in Britain, North America, Australia, India, and Ceylon react as well to local house dust as to dust from other parts of the world." It is not surprising, therefore, that Naranjo finds house dust to be more important than mold spores or pollens in producing positive skin reactions in his patients in Quito and Cali. It would be interesting to know if this is true also in the Oriental tropics.

Food sensitivities and idiosyncrasies were probably recognized long before other forms of allergic distress. It is the custom in virtually all textbooks on allergy to credit the Roman poet Lucretius (96 to 55 B.C.) with the first crystallization of current thinking on this subject when he observed tersely that "One man's meat is another man's poison." Unfortunately, less is known about the occurrence and extent of food allergies than of any other type of sensitivity reaction in tropical or temperate zones. As these are generally self-limiting, only the more extreme instances come to the attention of medical authorities; therefore, their incidence has not been determined. It is clear from the literature that allergic responses of a more or less severe or even transient nature may occur or develop with reference to almost any food anywhere. This has probably meant in the past that food allergies

in the warm climates would be attributable to the special cereals, fruits, nuts, and meats native to the equatorial belt. With the modern technologies of preservation, canning, dehydration, and freezing of foods plus transport by milk tanker and air freight it is evident that the foods originating within both temperate and tropical environments are being disseminated on an increasing scale throughout the world. One would think that if there ever were any significant differences in the nature and frequency of allergic disease due to tropical vs. temperate zone foods, these will reach an equilibrium in due course.

Thus far I have scrupulously avoided reference to the allergic phenomena which are doubtless of greatest professional interest to this audience, namely, those occurring in conjunction with parasitic and infectious processes in the tropics. I have done this because I assume that the majority of you are as well or better informed than I about these manifestations; they have been widely discussed and journalized. Secondly, up to this point I have arbitrarily limited myself to considerations of sensitivity as a primary cause of disease, one which appears to be eluding the attention of tropical medicine authorities. But for the record, let me remind you of such allergic sequellae as urticaria associated with malaria and amebiasis; dermatitis with schistosomiasis; the inflammatory structural damage and symptoms with tissue-inhabiting nematodes and trematodes; asthma with ascariasis; anaphylaxis with echinococcosis. These and a host of other such unholy alliances have added materially to the burden of illness where these maladies are endemic. Furthermore, many of the drugs used in the treatment of these diseases have been notorious in provoking allergic reactions, ranging all the way in severity from mere annoyance to fatal outcome. Perhaps the most conspicuous example is that of quinocrine hydrochloride used during World War II in the Army and Navy for mass prophylaxis, suppression, and treatment of malaria. The total number of resulting dermatoses, some of them fatal, probably will never be known (Nisbet, 1947). The proportion of soldiers dying from aplastic anemia was markedly higher among troops on extended atabrine suppressive therapy than among those who took no atabrine (Custer, 1946).

Are the allergic diseases and the allergic sequellae of the tropics important enough to be identified as a fourth category along with parasitic, infectious, and nutritional disorders? My gleanings from the literature are by no means conclusive, but they leave me with the following impressions.

Allergic disease, collectively, is probably less important as a health problem in the tropics than in the temperate zones, especially in this hemisphere. This is due not only to the reasons given above, namely, the presence in this country of highly antigenic species of ragweeds, the vagaries of tropical seasons, and the air-cleansing effectiveness of tropical rains, but also to alleged constitutional differences in the populations of these two zonal areas. All observers agree that there is less evidence of respiratory sensitivity among the indigenous races of the tropics than among others, but measurements of this difference are not adequate.

For 5 years Derbes and Engelhart (1943) surveyed hospital patients admitted for asthma at Charity Hospital in New Orleans and found that Negroes had lower rates per 1,000 admissions, 4.04 for males and 3.8 for females, than did white residents, 7.0 for males and 6.4 for females. Scheppegrell (1923) found that hay fever was about three times as frequent in whites as in Negroes in Louisiana. Skin tests, which are not necessarily indicative of frank allergic disease when positive, were used by de la Riva to measure pollen sensitivity in 53,120 persons in Cuba. He found insignificant differences in positive reactions between white Cubans (0.03% of 27,950) and Negro Cubans (0.0% of 1150) but somewhat larger ones between them and Europeans (0.68% of 16,880) and Hebrews (3.12% of 7140) residing in Cuba. What these differences really mean is hard to visualize, as it must be extraordinarily difficult, in either temperate or tropical environments, to obtain statistically adequate samples of comparably exposed population groups differing only in racial composition.

However, even if the occurrence of allergic morbidity is racially distinctive or is less frequent in native than in nonnative residents of the tropics, there is evidence, as indicated above, that indigenous populations are affected to a sizeable, even if undetermined, degree. With approximately three-quarters of a billion persons (roughly, a third of the world's inhabitants) estimated to live in the tropics where facilities for the diagnosis and therapy of allergic disorders

are miniscule and beyond the reach of most people, it is reasonable to assume that the problem is of much greater dimensions than we are aware.

If this assumption is substantively correct, why have the diseases of allergic etiopathogenesis been disregarded in so much of our research, our writings, and our oral dissertations about tropical medicine? I suspect that this may stem from our relative lack of knowledge or awareness of allergic processes and illnesses because, as I mentioned above, our training and experience have focused on the traditional infectious and parasitic diseases of the tropics. It is trite to observe that people find it so much easier to travel on well-established, familiar highways that they are reluctant to explore new paths and byways. Add to this the preoccupation of the too few physicians in the tropics with the more obvious medical problems and emergencies, and the reasons for the meagerness of our basic and practical information about tropical allergies become more understandable.

These considerations lead me to suggest that our Society initiate steps to develop more interest and knowledge concerning these diseases and complications as a segment of the total spectrum of tropical medicine. This would involve, first of all, the stimulation of fundamental and applied research in the laboratory, in the field, and in the hospital, to define, to understand, and to meet effectively the problems of allergy and allergic disease wherever they occur. In particular, those aspects pertaining to the tropics should be explored and studied until we possess a more extensive and competent understanding of tropical allergy and allergens than I have been able to put before you this evening. Lastly, we should see that the information thus acquired is disseminated widely by means of papers and symposia presented before this Society and other interested organizations, and is incorporated into textbooks on tropical medicine and medical school curricula.

In conclusion, I believe that in my somewhat overambitious effort to summarize information concerning tropical allergies and allergens, I may have been more successful in calling attention to the paucity of existing knowledge in these fields. It is my earnest hope that the members of this Society will recognize from this presentation the real need for evaluating the role of allergy in tropical disease.

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STATISTICAL EVALUATION OF THE FORMALIN-ETHER (406TH MGL) FECAL SEDIMENTATION CONCENTRATION PROCEDURE[1]

DALE E. WYKOFF[2], LYMAN P. FRICK[3] AND LAWRENCE S. RITCHIE[4]

406th Medical General Laboratory, APO 343, San Francisco, California

INTRODUCTION

The formalin-ether (406th MGL) sedimentation concentration procedure (Ritchie, 1948) has proved to be a valuable aid for the detection of intestinal parasitoses. Its simplicity, flexibility and economy make it suitable for use in laboratories handling either a few or large numbers of stool specimens. At the 406th Medical General Laboratory in Japan, the procedure has been used for thousands of routine clinical and survey examinations. Furthermore, the procedure appears to have received approval in other laboratories. For these reasons, it is believed that the procedure merits a critical evaluation.

Preliminary evaluations of the formalin-ether procedure for the detection of various intestinal parasites have been reported by Wykoff and Ritchie (1952) and Ritchie, Pan and Hunter (1952, 1953). The latter investigators adequately demonstrated that under the conditions encountered in Japan the procedure was equal or superior to the standard, $ZnSO_4$ centrifugation-flotation technic as used by Faust et al., (1938) or Tobie et al., (1951). After determining the relative efficiency of the formalin-ether procedure as compared with a standard technic, it became desirable to arrive at mathematical expressions for the probability of detecting specific parasite objects on the first examination with the procedure. This evaluation of technic efficiency, which constitutes the subject of this report, was accomplished by statistical treatment of data that were obtained in several, extensive, multiple stool examination series.

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[2] Address Dept. of Medical Zoology, Walter Reed Army Institute of Research, WRAMC, Washington 12, D. C.

[3] Address Parasitology Branch, Sixth US Army Area Medical Laboratory, Fort Baker, California.

[4] Tropical Research Medical Laboratory, APO 851, New York, New York.

PROCEDURES

In 1952 an extensive investigation of the epidemiology and chemotherapy of amebiasis was started in four rural villages of Yamanashi Prefecture, Honshu, Japan. Approximately 1,000 people were involved in the study and all or part of them were examined for intestinal parasites on a number of occasions during the course of the next several years. The statistical evaluation reported herein was based on five-stool-examinations series performed in January and September, 1952, when all persons were examined. Subjects in two of the four villages were also examined in a five-stool series in March, 1952. All data were used in the supplementary evaluation. Cooperation by the examinees in all of the series was nearly complete; the few omissions that did occur in one series or another were about equally divided between infected and non-infected persons.

Stool specimens were passed normally and collected in waxed-paper sputum cups on alternate days within a 10-day period. Two periods of 25 days each were allotted for the January and September surveys and all examinations were made under semi-field conditions on a laboratory train operated by the 406th Medical General Laboratory. Because of the delay involved in delivering the specimens from village to train, direct-smear preparations were generally omitted. All specimens were examined by the formalin-ether technic. The technic can be modified at several points but, as used in this study, the procedure given by Ritchie (1948) and Ritchie, Pan and Hunter (1953) was followed. Usually one or more technicians prepared the specimens in lots of 12 and the concentrates were examined by a group of five highly qualified microscopists. One or two technicians assisted with the examinations at different times, but the vast majority of all examinations were performed by the basic group. Except in a very few instances, each specimen was treated as an "unknown";

that is, each examination in a series on an individual was made independently of findings from any previous examination. Identification of protozoans was based on cysts since trophozoites are destroyed in the procedure. In addition to the normal examination of formalin-ether concentrates, the microscopists also made approximations of parasite burdens, and assisted with the tabulation of results and other tasks incidental to the surveys. Their normal work load averaged 40 to 50 examinations per day.

Estimations of the efficiency ($\hat{E}$) of the technic and of the prevalence ($\hat{P}$) of the various infections were computed by the method of maximum likelihood, using formulas derived by Sawitz and Karpinos (1942) and modified by Mantel (1951). Approximations of standard errors of estimate for $\hat{E}$ and $\hat{P}$ were obtained by formulas given by Mantel (*loc. cit.*). Although $\hat{E}$ in reality represents the probability of finding an organism in a single examination, its value is commonly expressed in per cent, and the terms "probability" and "efficiency" are stipulatively used synonymously. For convenience, this usage is followed in this report and the terms "efficiency", "$\hat{E}$ value" and "estimator of efficiency" are all used interchangeably.

RESULTS

In Table 1 there is summarized the mean efficiency of the formalin-ether technic for the detection of five types of protozoan cysts and helminth ova, together with appropriate estimates of standard error. These data were obtained from the January and September examinations. All individuals were examined in both series but, since most of the people with protozoan infections in two villages were treated after the January series and there was a paucity of some infections in other villages, a part of the data was not used in the computation of some of the means. There were also three other omissions of data which will be explained later.

Cysts of *Entamoeba histolytica* and *Endolimax nana* were detected with almost equal facility, mean efficiencies being, respectively, 60.2 and 60.4 per cent. The $\hat{E}$ value for *Entamoeba coli* (75.1%) was the highest for any protozoan while it was lowest for *Giardia lamblia* (44.5%).

Eggs of the three common helminths were detected readily by the procedure. Mean efficiencies for *Ascaris lumbricoides* and *Trichuris trichiura* were 91.9 and 92.2 per cent, respectively and, for hookworm, 78.9 per cent. The formalin-ether technic was considerably less effective for *Schistosoma japonicum* and *Trichostrongylus* sp., whose $\hat{E}$ values were, respectively, 47.6 and 41.1 per cent.

TABLE 1

Estimated mean efficiencies of the 406th MGL fecal concentration procedure for the detection of various intestinal protozoan and helminth parasites; comparison with data of Tobie et al. (1951) *for zinc sulfate centrifugation flotation technic*

Parasite	Efficiency and SE* in per cent	Tobie *et al.* data
Entamoeba histolytica	60.2 ± 1.5	59 ± 2
Entamoeba coli	75.1 ± 0.7	76 ± 2
Endolimax nana	60.4 ± 1.1	65 ± 2
Iodamoeba bütschlii	55.7 ± 2.4	61 ± 3
Giardia lamblia	44.5 ± 1.3	38 ± 4
Ascaris lumbricoides	91.9 ± 0.4	
Trichuris trichiura	92.2 ± 0.4	
Hookworm	78.9 ± 0.7	
Trichostrongylus sp.	41.1 ± 1.4	
Schistosoma japonicum	47.6 ± 1.2	

* Standard error of estimate.

Included also in Table 1 are data obtained by Tobie *et al.*, (1951) on the efficiency of the $ZnSO_4$ centrifugation-flotation technic for the detection of the same protozoan cysts. Differences between these figures and those of the present report cannot be tested statistically because a different method of analysis was used in each study; however, it would appear that the two technics were almost equally efficient for the detection of cysts.

In Table 2 a comparison is made between the efficiency of the formalin-ether technic in the detection of parasitic infections in four population groups and, where applicable, in two series of examinations. It is evident that the efficiency varied rather widely for some organisms, and insofar as they could be tested statistically, some of the differences were probably significant. In such instances, certain omissions of data were believed to be advisable when the mean efficiencies in Table 1 were computed. For example, $\hat{E}$ values for *E. histolytica* ranged from 59.4 per cent in village M to 71.7 and 67.3 per cent in village H (January vs. September). The difference between the low value and either of the two

TABLE 2

Estimates of efficiency of 406th MGL fecal concentration procedure and prevalency of intestinal parasitoses in four rural Japanese villages

Parasite	Village "T"		Village "M"		Village "K"				Village "H"			
	January		January		January		September		January		September	
	$\hat{E}$	$\hat{P}$	$\hat{E}$	$\hat{P}$	$\hat{E}$	$\hat{P}$	$\hat{E}$	$\hat{P}$	$\hat{E}$	$\hat{P}$	$\hat{E}$	$\hat{P}$
Entamoeba histolytica	60.0	14.2	59.4	27.8	61.6	22.4	60.3	19.3	71.7	17.3	67.3	20.9
Entamoeba coli	73.8	42.2	72.6	49.9	76.9	57.1	73.8	58.8	78.8	51.8	74.4	57.0
Endolimax nana	64.8	32.4	54.7	33.1	63.1	35.4	57.8	35.2	71.3	39.3	62.4	45.9
Iodamoeba bütschlii	—	—	56.4	9.1	60.7	6.3	46.8	6.3	62.4	5.5	50.7	6.7
Giardia lamblia	62.8	10.4	47.6	17.3	44.1	17.2	40.5	20.8	46.8	5.7	43.7	7.7
Ascaris lubricoides	90.6	74.8	89.5	82.7	95.5	82.8			92.6	81.6		
Trichuris trichiura	88.1	76.9	91.4	93.2	97.2	93.7			92.4	93.7		
Hookworm	82.5	68.6	76.6	59.8	80.8	65.6			75.7	61.2		
Trichostrongylus sp.	48.4	27.3	34.5	30.3	44.5	29.8			37.8	21.1		
Schistosoma japonicum	46.7	57.5	44.3	40.8	53.4	36.3			—	—		

TABLE 3

Cases added and missed by a five-stool examination series performed 56 days after an initial series. Villages "T" and "M". Examinations by 406th MGL procedure

Parasite	Infections in Jan. series	Cases added in Mar. series		Cases absent in Mar. series	
		No.	Per-cent*	No.	Per cent*
Entamoeba histolytica	128	4	3.0	†	
Entamoeba coli	264	15	5.4		
Endolimax nana	193	14	6.8		
Iodamoeba bütschlii	32	2	5.9		
Giardia lamblia	84	16	16.0		
Ascaris lumbricoides	448	36	7.4	27	5.6
Trichuris trichiura	487	15	3.0	11	2.2
Hookworm	359	23	6.0	26	6.8
Trichostrongylus sp.	153	47	23.5	52	26.0
Schistosoma japonicum	259	34	8.7	47	11.9

* Per cent of assumed January aggregate.

† Protozoan data not suitable for analysis because of treatment intervening between January and March examinations.

high ones is probably significant. Therefore, in order not to over-estimate the efficiency of the technic for *E. histolytica* cyst detection, data for village H were omitted in the computation of the mean efficiency. For this reason also, data for village H (January) were not included in the calculation of the mean efficiency for *E. nana*, and for village T for *G. lamblia*. All data were used for the computation of mean $\hat{E}$ values for *E. coli* and *Iodamoeba butschlii* and for all of the helminths.

Participants in villages M and T were re-examined in a five-stool series 56 days after the initial series, and the findings from the two series were compared. The number of additional infections found in the second examination series and the per cent of a total these figures represent are summarized in Table 3. Since it was not possible to differentiate between infections that might have been missed in the original series and new infections that might have been acquired in the interval between the two series, all additional infections were assumed to have been present in the initial series but to have been missed. In all instances, the number of additional infections was greater than the hypothetical number of undetected infections that could be calculated by the maximum-likelihood formulas (Sawitz and Karpinos, 1941); however, with the exception of the figure for *Trichostrongylus* sp., none of the differences was great enough to affect prevalency rates significantly.

The percentage of infections estimated to remain undetected after each examination in a hypothetical series of five made with the formalin-ether technic is illustrated by the bar graphs comprising Figure 1. All data were used for the

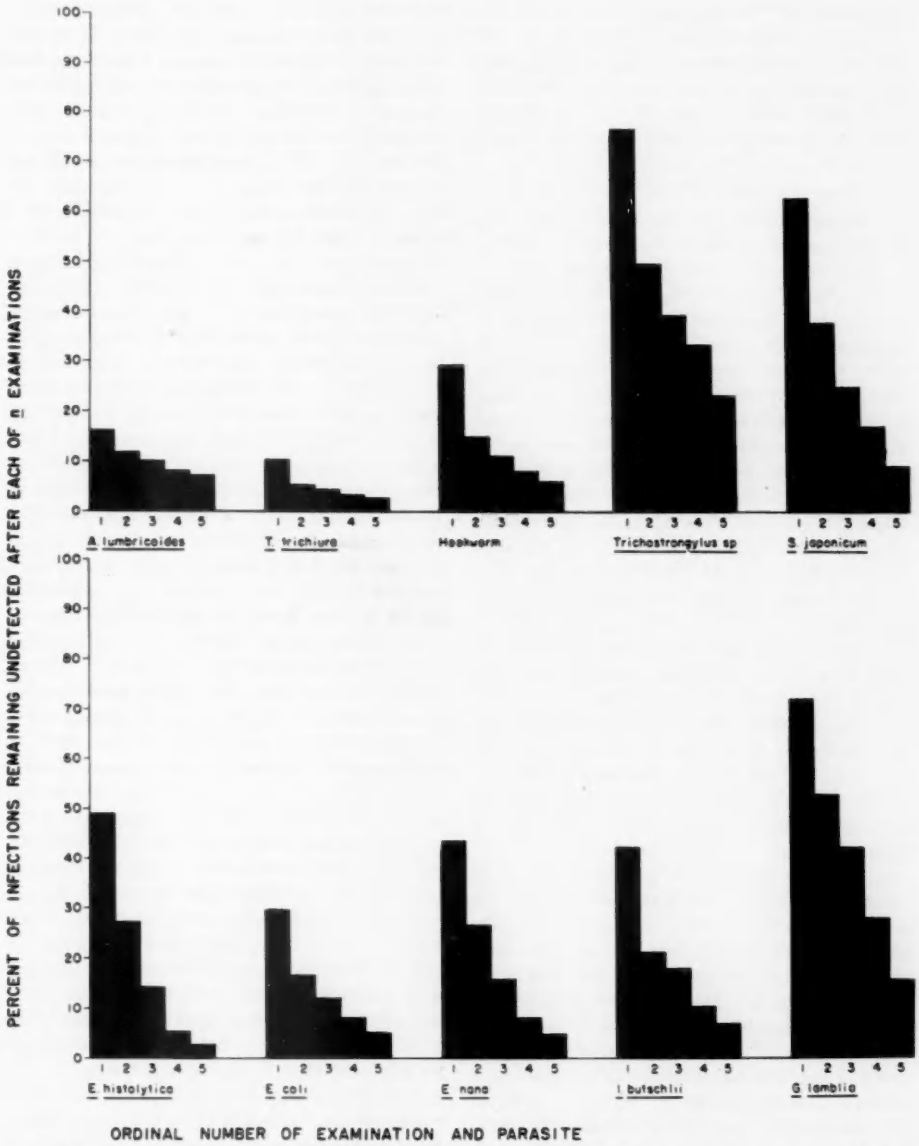

FIG. 1. Estimation of percentage of helminth and protozoan infections remaining undetected after each examination in a series of five made with the formalin-ether (406th MGL) procedure.

protozoans, while only data from the January examinations in villages T and K were used for the helminths. For the reason given in the paragraph above, the raw data for each species were adjusted by adding the per cent of additional infections found in the re-examination series (Table 3) in villages M and T. It is a reasonable assumption that the "miss rate" was probably

uniform in the several villages. If only the actual initial series of five examinations is to be considered, then the percentages given in Table 3 would be subtracted from each bar. The length of the first bar would then be inversely proportional to the computed efficiency of the technic.

DISCUSSION

Ritchie, Pan and Hunter (1952, 1953) have shown that the formalin-ether technic can concentrate parasite objects in stools and maintain the diagnostic integrity of such objects. These investigators compared the technic with the standard $ZnSO_4$ technic and reported that the relative efficiency of the formalin-ether procedure was consistently higher than that of the latter for all parasite forms studied. Since negligible distortion is produced in cysts and pre-cysts by the formalin-ether technic, it was possible to detect several times as many small-race *E. histolytica* infections with this technic as with the flotation procedure. The difference between relative efficiencies for large-race *E. histolytica* or other cysts was less, although of diagnostic importance.

On the basis of an examination of part of the data used in the present report, Wykoff and Ritchie (1952) estimated that the efficiency of the formalin-ether technic for the detection of *E. histolytica* cysts (races disregarded) was approximately 60 per cent. The complete analysis shows that this estimate is valid and that the efficiency for other cysts and ova is commensurate with this figure.

It was not possible in this study to make parallel tests with the formalin-ether and $ZnSO_4$ technics. Neither are the authors aware of any study in which the efficiency of the latter procedure has been determined by the maximum-likelihood formula. The estimates of efficiency reported for the technic by Tobie *et al.*, (1951) were computed by a different method, hence it is not appropriate to test statistically the differences between those values and the ones given in Table 1 of the present report. It is probable, however, that the two procedures would prove to be comparable in efficiency regardless of the method by which the efficiencies were determined.

In any series of stool examinations it is expected that a variety of sources of experimental error will be encountered which will affect the results of those examinations. These sources of error are included in the two assumptions that must be made when technic efficiencies are determined by statistical methods. The assumptions are: (1) that the examination procedure does not yield false positives, and (2) that there is equal probability of detecting any particular type of parasite object in all individuals who may be infected with that form. It is also implied in the latter assumption that the parasite object in question must be of a type that is capable of being detected by the examination procedure. Obviously, the validity of estimates of efficiency that are determined by a particular statistical procedure will be limited by the extent to which the procedure will adjust for these assumptions.

Adjustment for seeming false positives can be made in the method of analysis used by Tobie *et al.*, (1951) or in the maximum-likelihood method, by the use of appropriate formulas for the computation of standard errors of estimate for $\hat{E}$ (Mantel, 1951). These formulas take into consideration the number of positives that occur only one time in a series and which, therefore, may be due to error. In theory, neither the $ZnSO_4$ technic nor the formalin-ether technic yields false positives. Parasite objects are either in the concentrates or they are absent. If present, the only limitation to their detection and identification is the proficiency of the examiner. In actual practice, however, and especially in multiple-stool examinations, instances do occur which, in retrospect, suggest the occurrence of false positives. These do not occur frequently and simple explanations can be offered for their occurrence; nevertheless, it is appropriate that adjustment should be made for them.

The assumption of equal probability of detection introduces the problem of false negatives and is considerably more complex. It has long been recognized that negative findings may commonly occur in examinations on persons who actually are positive, and it is for this reason that the efficiency of an examination procedure cannot be calculated from the simple ratio of number of positive findings to number of examinations made. False negatives may occur for a number of reasons. Some are common to examinations for both protozoans and helminths and involve only technical error. These include the relative proficiency of technicians, pressure of a heavy work load and other working conditions, over-emphasis on detection of one form or another, and perhaps

the manner of sampling from a specimen. Other reasons for false negatives are perhaps more pertinent to examinations for protozoans. Included here are such factors as cyclical or spasmodic occurrence of the objects in stools, possible biologic differences within strains, and the effect that the nature of the stool itself may have on detectability of certain objects. But the single, most important cause of false negatives is the variation in intensity of infection, or parasite burden, that occurs between one infected individual and another, or even in one individual at different times. There is a basic limitation to the sensitivity of either the $ZnSO_4$ or formalin-ether technics for any particular type of cyst or egg; furthermore, this limitation may be affected by the intervention of any of the variables listed above. In view of this, it is to be expected that a certain proportion of infections will always be missed.

If sufficient examinations are performed so as to insure detection of at least the bulk of the infections, it is possible, on the basis of those data, to make a reasonable prediction of the proportion of positives that will be missed. Assuming that this can be done, then adjustment can be made for false negatives in either method of analysis. However, because of the number of variables involved and their interaction with one another, it is unlikely that adjustment is ever complete or that adjustment for forms such as protozoan cysts is equivalent to that for helminth ova. This constitutes the principal disadvantage to the use of either statistical procedure. However, since a better procedure is lacking, the deficiencies of the available ones can only be recognized and the mathematical expressions of efficiency obtained with them weighed accordingly.

Despite the discussion above, the differences between estimates of efficiency observed in different population groups in this study and the one by Tobie *et al.*, (1951) are assumed to be real and not due to chance alone. In the available methods of analysis, the magnitude of the $\hat{E}$ value is a function of rapidity or regularity of detection of positives. This in turn can be a function of infection intensity, and recognition of the fact that the mean intensity of infection in one population group can be much higher than in another is sufficient explanation for a significant difference between $\hat{E}$ values in the two populations. Tobie *et al.*, have suggested that there may also be seasonal differences in parasite burdens. However, there was relatively little evidence of this in the present study. The possibility that strain differences may occur within a species and that these may affect the detectability of the strain should also be considered. In such an instance, the detectability of strains as reflected in lower or higher estimates of technic efficiency could function independently of the prevalency of the strains. In a study of experimental *E. histolytica* infections in prison volunteers, Beaver *et al.*, (1956) observed that the overall efficiency of the $ZnSO_4$ technic combined with direct-smear procedures was 86 per cent, as compared with a figure of approximately 60 per cent observed by Tobie *et al.*, (1951) and Mackie *et al.*, (1955). The high efficiency was attributed in part to biologic characteristics of the strain, as well as to the great uniformity of diet and living conditions of the volunteers. Any of these factors or combinations of them could have been in operation in the present study. The four villages are well separated geographically and are on different watersheds. There is comparatively little intercourse between villages, and the manner of living and occupations of the inhabitants are not conducive to exchange of parasites between villages.

It has been pointed out by Tobie *et al.*, (1951), and it is also true in the present study, that for practical use the efficiency rate of a technic is expressed as though there were hypothetically uniform efficiency and prevalency rates. These, of course, do not exist; hence the efficiency rate becomes a function of many variables peculiar to a particular population or set of conditions, and is therefore true only for that situation. However, if a mean efficiency rate is determined by appropriate methods, the fact that it is only an average does not detract from its practical use as an indicator of the value of the procedure.

There is little doubt that the probability of detecting intestinal parasites increases with the number of examinations actually performed; however, there are several opinions concerning the number of examinations that should be made to insure detection of all infections. A number of studies have been made with *E. histolytica* in this respect and an excellent review of the problem is given by Anderson *et al.*, (1953). For the purpose of the present study, it was assumed that five examinations performed on alternate days would be a practicable schedule and that all but

a minimal number of infections would be detected. Five examinations over a longer interval, e.g., a month, might afford a higher efficiency since the longer interval would tend to circumvent biologic fluctuations in cyst production. Additional infections were detected in two villages when examinations were repeated after 56 days, but the number of such cases was not great. These cases include those that might actually have existed at the time of the first series but which were missed, as well as those that might have been acquired in the period between the two series. Most of the additional protozoan infections probably fall into the first category since the acquisition rate for these infections is not high in these villages (unpublished data). A larger number of the additional *Ascaris* infections, and by inference the *Trichuris*, hookworm and *Trichostrongylus* cases, possibly represent newly acquired infections since there is an abrupt increase in the incidence of ascariasis in this area in the early part of the year (Clin. Lab. and Res. Rep., 406th Med. Gen. Lab., 1953). The additional schistosomiasis japonica cases probably were misses. Since it was not possible to differentiate between missed and newly acquired infections in the reexamination series, all of the additional cases have been treated as missed cases. This has the effect of lowering the computed efficiency of the formalin-ether technic by an amount equal to the proportion of additional infections. The true efficiency of the technic, therefore, probably lies between the computed mean values (Table 1) and that indicated by the inverse value of the first bar in the graphs comprising Figure 1.

The miss rate for *E. histolytica* (3%) was probably somewhat too low. It was the parasite of principal interest, consequently somewhat more intensive efforts were made to detect it. Excessive concentration on cysts during microscopy can result in larger objects such as ova being overlooked. Also, some investigators are of the opinion that helminth ova often can be found more readily in direct smears than in concentrates (Beaver, personal communication).

These observations do not answer the question of how many examinations should be performed in order to detect all infections in a population. They do, however, give a good approximation of the proportion of positives that might be expected to be found in any series of examinations up to five in number.

Ordinarily the selection of a fecal concentration technic for routine use is determined by personal preference and training. After more than 8 years of experience with the formalin-ether procedure in the parasitology section of the 406th Medical General Laboratory in Japan, the procedure has been found to be a highly effective and reliable method for the detection of all common intestinal parasites. For forms such as *S. japonicum*, the MIFC procedure (Blagg *et al.*, 1955) is probably the method of choice. The formalin-ether procedure is simple in its mechanics and requires no unusual equipment or supplies. The only item of appreciable cost is ether.

As the procedure is performed in this laboratory, an excellent sampling of the stool is afforded by the practice of partially comminuting the entire specimen in its container. Ordinarily, water is used for comminution; however, 7.5 or 10 per cent formalin may be used if desired. Specimens may be prepared and examined singly or in groups. After formalin has been added to the washed and strained specimen, it may be stored for varying periods; also, concentrates have been stored at 5°C for long periods without harm to cysts and ova. No evidence has been found to suggest that prior formalinization affects the recovery of parasite objects by the formalin-ether technic. For that reason the procedure is ideally suited for surveys.

Because of the nature of the two technics and the different principles involved (sedimentation vs. flotation), occasions often arise when one of the procedures is better suited for use with certain stools than is the other one. For this reason, the formalin-ether technic should be considered as a complement to the $ZnSO_4$ procedure rather than as a replacement.

SUMMARY AND CONCLUSIONS

Estimations of efficiency of the formalin-ether sedimentation concentration procedure for the detection of ten common intestinal parasites have been determined from an analysis of data obtained in multiple-stool examination series. Approximately 1,000 residents of four, rural, Japanese villages were examined in two five-stool series and findings were analyzed by the maximum-likelihood method of Sawitz and Karpinos. Mean estimates of efficiency for the detection of protozoan cysts were comparable to those computed by other investigators for the $ZnSO_4$

centrifugation-flotation technic. These estimates, in per cent, for the formalin-ether procedure were 60.2 for *Entamoeba histolytica*; 75.1 for *E. coli*; 60.4 for *Endolimax nana*; 55.7 for *Iodamoeba bütschlii*; and 44.5 for *Giardia lamblia*. Corresponding figures for helminths were: 91.9 for *Ascaris lumbricoides*; 92.2 for *Trichuris trichiura*; 78.9 for hookworm; 41.1 for *Trichostrongylus* sp.; and 47.6 for *Schistosoma japonicum*.

The efficiency of the formalin-ether procedure for the detection of some organisms, especially protozoan cysts, was observed to vary and, in some instances, the differences were probably significant. These variations are assumed to be due to a combination of factors including technical error, limitations of the analytical method and the examination procedure itself, possible biologic characteristics of the organisms that might affect their detection, and normal fluctuations in the mean infection intensity in the different population groups.

Re-examination of about one-half of the people within 60 days after the initial examination series permitted a better estimate of the reliability of the technic. The number of additional infections detected in the second examination series was about equal in all instances to the number of cases present in the first series, but absent in the second. The rate of misses for *E. histolytica* was no greater than the rate for more easily recognized forms. On the basis of the latter data, it was estimated that examination of five separate stools by the formalin-ether technic will detect about 97 per cent of amebiasis cases in a population. Similar data were also obtained for the other nine parasites.

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EXPERIMENTAL STUDIES ON *ENTAMOEBA HISTOLYTICA* IN KITTENS

SISTER M. ANN JOSEPHINE, C.S.C.

Department of Bacteriology, College of Medicine, University of Utah, Salt Lake City

The most important parasitic ameba of the alimentary tract is *Entamoeba histolytica*. Although this protozoan is primarily a parasite of man and monkeys, it can also infect a number of other mammals including the dog, rat, kitten, guinea pig, and rabbit. Cysts are not usually found in experimental infections of cats or dogs; they are formed, however, in the naturally occurring infection in man and monkeys and often in rats.

Experimental animal amebiasis has contributed greatly to the study of the human disease and to an understanding of the character of the ameba. As reported in the literature, the type of infection varies with the animal used. In the monkey, experimental infection is invariably mild and chronic. Canine amebiasis varies in its severity, its acuteness or chronicity depending on the age of the animal, its nutritional status, and the way in which it is preconditioned for infection. Rabbits, guinea pigs, and rats develop a nonprogressive infection which, in the case of rabbits and rats, heals spontaneously. In the kitten, amebiasis occurs in a rapidly progressive and fatal disease. The type of animal used, however, has not been uniform and many of the kittens brought to the laboratory have undoubtedly been infected with other types of parasites prior to experimental infection with *E. histolytica*.

In the present investigation the infection of kittens with *E. histolytica* of human origin was undertaken to determine whether infection with the parasite per se is sufficient to account for the acute form of feline amebiasis or whether the difference in degree of symptoms may be influenced by factors such as the psycho-physical response to stress, nutritional status of the animal, and intercurrent infection, resulting in an overwhelming infection in a species of animal which under more normal conditions may be only moderately susceptible to the ameba.

Extending the work of Comer *et al.*, (1956) who studied the protein fractions in the plasma of normal dogs as compared with the plasma of these animals during active infection with *E. histolytica* and after spontaneous cure, information was also collected on the effects of infection and active immunization on the serum proteins of kittens.

MATERIALS AND METHODS

All experimental cultures were inoculated from the laboratory stock culture of the K-9 strain[1] of ameba grown with mixed bacterial flora. These cultures were propagated on Ringer's egg-serum medium to which rice starch was added.

Experimental animals. Newly weaned kittens (5 weeks) were obtained locally; both sexes are represented in the series. The animals were placed in cages as soon as they were brought to the laboratory, and stool specimens were examined for parasites by the direct smear and $ZnSO_4$ centrifugal-flotation technic. If parasite-free on two examinations, the kittens were used in experiments. Animals harboring intestinal protozoa or worm eggs were not used. Litter mates were distributed as equally as possible, and all kittens were separated into two groups. Animals in Group I were maintained on canned cat food and kept in separate cages; Group II animals had their diet supplemented with fresh cooked meat and canned milk and were permitted the freedom of a large outdoor cage. Control animals were housed in separate cages. Kittens whose behavior appeared abnormal during the period of observation were not used in the experiment. When aninals belonging to Group II gave evidence of illness, they were placed in separate cages.

Production of experimental infection. Animals were anesthetized with pentobarbital sodium given intraperitoneally and infections were induced following laparotomy. Five ml of 48-hour cultures of *E. histolytica* trophozoites were inoculated directly into the cecum after the lower portion of the rectum had been tied off. Previous counts on similar cultures ranged from 500,000 to 750,000 amebae per ml. The abdominal wall was closed with Michel clips and 0.5 ml of Combiotic was injected intraperitoneally. Control animals

[1] Kindly furnished by the Communicable Disease Center, Chamblee, Georgia.

were inoculated using the same technic and identical amounts of the bacterial flora, but without amebae, from 24–48 hour cultures in Ringer's egg-serum medium.

Kittens were sacrificed at different periods of time after infection by means of ether and autopsies were performed immediately. Blood was obtained by cardiac puncture for complement-fixation tests and electrophoretic analyses. Intestinal lesions were located according to the procedure outlined by Rees (1929).

Production of anti-serum. Anti-amebic serum was produced in kittens by the inoculation of trophozoites intraperitoneally. Each series consisted of six inoculations, at 3-day intervals, of 1.0 ml of pooled 48-hour cultures of washed trophozoites and 0.5 ml of Combiotic. Each inoculum consisted of 1 to 3,000,000 organisms. Seven days following the final inoculation of organisms, blood was obtained by cardiac puncture and the serum stored in the deep freeze at −15°C. Food was withdrawn from animals the evening prior to the day of bleeding.

Complement-fixation tests. The micro-Kolmer complement-fixation technic was employed in the present studies. Eli Lilly *E. histolytica* Antigen, lot number P-58225[2] at a 1:5 dilution was used in each series.

Electrophoretic analyses. All sera were analyzed by the zone electrophoretic technic using the Spinco apparatus. Barbiturate buffer at pH 8.6 and 0.1 ionic strength was used throughout this study; separation was allowed to proceed for 16 hours at 5 milliampers per cell constant current. Strips were quickly oven-dried to fix the pattern and bands were made visible by staining. Separated serum proteins were evaluated densitometrically. Concentrations of the total protein nitrogen were derived by the micro-Kjeldahl technic. Since the experimental design included young animals of different ages, proper account of this variable was necessary (Moore, 1945). A pool of blood sera from uninoculated, healthy, parasite-free kittens of different age groups was analyzed electrophoretically and normal values established for comparsion with experimental data (Table 1). This series is being supplemented at the present time.

[2] This antigen was kindly supplied by Dr. James B. Hammond, Lilly Laboratory for Clinical Research.

TABLE 1

Electrophoretic analyses of the blood serum proteins of normal kittens

No. animals	Age of animals (wks.)	Albumin (per cent)	Globulins (per cent)				A/G ratio
			Alpha 1	Alpha 2	Beta	Gamma	
5	5	55.4	5.4	18.9	13.5	6.7	
4	5	50.0	6.2	24.3	13.8	5.5	
7	5	52.0	4.3	21.6	15.3	4.0	
Average	5	52.5	5.3	21.6	14.2	5.4	1.1
5	7	40.2	7.9	26.3	13.5	11.9	
4	7	38.8	5.9	26.5	15.9	12.6	
6	7	43.1	4.2	22.6	16.2	10.6	
Average	7	40.7	6.0	25.1	15.2	11.7	0.7
5	10	41.2	4.0	18.4	13.2	23.2	
5	10	39.7	5.1	18.9	15.2	21.8	
5	10	32.9	7.2	20.3	14.3	24.2	
Average	10	37.9	5.4	19.2	14.2	23.1	0.6
4	12	37.7	1.7	18.6	15.6	25.9	
5	12	39.4	5.3	14.2	13.6	26.2	
Average	12 (mo.)	38.6	3.5	16.4	14.6	26.1	0.6
4	6	37.2	6.1	15.4	11.2	29.1	
3	7	36.9	7.6	12.4	10.5	31.3	0.5

TABLE 2

Summary of macroscopic findings at autopsy of kittens inoculated intracecally with Entamoeba histolytica, *K-9 strain*

Description of animals:	Group I*	Group II†
Inoculated	48	20
Died before 24 hours	5	0
Studied for lesions	43 (100%)	20 (100%)
From which amebae recovered	26 (60%)	12 (60%)
With definite macroscopic lesions	21 (49%)	4 (20%)
With suggestive lesions	8 (19%)	4 (20%)
With no macroscopic lesions	14 (32%)	12 (60%)

Five animals inoculated with bacterial flora: no deaths; no suggestive lesions.

* Animals confined to separate cages and maintained on canned cat food.

† Animals housed in large outdoor cage and maintained on canned cat food supplemented with fresh meat and milk.

RESULTS

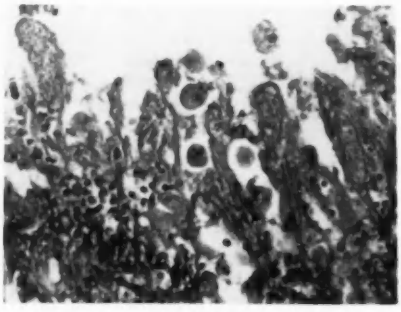
Ninety-nine kittens were employed in the present investigation; of these, 79 were experimentally infected with *E. histolytica*. Fecal diagnoses made on the day the animals were bled and/or examined for amebic lesions revealed that 8 per cent of the experimentally infected kittens found parasite-free at the beginning of the experiments had developed coccidiosis. Of the six animals infected with *Isospora* five developed macroscopic lesions following intracecal inoculation of *E. histolytica*. These animals are not included in the data presented in Table 2.

Following experimental infection the kittens appeared to experience considerable discomfort because of the intestinal obstruction created by the ligation of the rectum. Continual straining in an attempt to evacuate the bowel frequently loosened the suture and many of the inoculated animals began to pass dysenteric stools on the third post-operative day.

Animals from both groups were sacrificed at different periods of time and examined for amebic lesions. Infection with *E. histolytica* followed more frequently in frightened, nervous animals confined to separate cages during the course of the experiment than in those kittens with placid dispositions and good eating habits. Animals that accepted food during the course of the infection survived for longer periods of time than those that refused to eat following experimental infection. There was a significant decrease in the incidence of infection in kittens permitted to associate with other animals in a large outdoor cage and maintained on a supplemented diet as compared with animals placed in separate cages and maintained on canned cat food (Table 2).

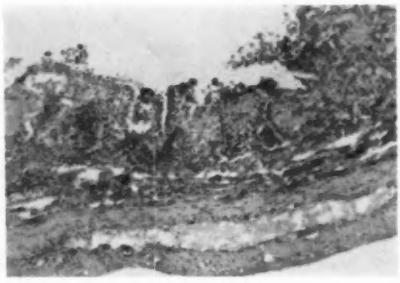
Extensive ulceration of the intestine terminating in a fulminating infection was evident in three kittens only in the experimental series (kittens 8, 9, and 10 of Group I). Areas of hyperemia were observed in seven kittens 5 days

(A) Actual Size



(B) × 200

(C) Actual Size



(D) × 100

FIG. 1. A, Section of colon of kitten 16 showing multiple amebic ulcers. B, *Entamoeba histolytica*, K-9 strain, invading gland crypts and the interglandular stroma of the superficial mucosa of the large bowel of kitten 16. C, Section of colon of kitten 10 showing lesions of acute amebic dysentery. D, Amebic ulcer with undermining of the mucosa and submucosa of kitten 10.

TABLE 3

Electrophoretic analyses of the serum proteins of kittens

Number examined	Age (wks)	When exam. (da. post inoc.)	Albumin (per cent)	Globulins (per cent)				C-F test*
				Alpha 1	Alpha 2	Beta	Gamma	
7	6	5	50.0	3.3	28.0	6.6	12.0	Neg
4	6-7	10	46.3	3.6	30.1	9.5	10.2	Neg
8	7	12	48.3	1.8	29.6	11.7	8.4	Neg
8	7-8	15-17	33.7	5.6	33.5	14.8	12.0	Neg
5	10-12	35-40	49.1	2.1	19.1	19.1	22.9	Neg
8	8	Normal	42.1	4.5	20.5	14.7	16.9	Neg
12	8	Hyperimmune	40.4	3.5	20.5	11.2	24.3	Pos

* C-F = complement fixation test.

following experimental infection, and areas of hyperemia with a few suggestive lesions were found in 8 of the 12 kittens sacrificed on the tenth post-inoculation day. Eight of the 10 animals sacrificed on the twelfth, and 14 of the 20 animals sacrificed on the fifteenth and seventeenth post-operative days exhibited definite macroscopic lesions (Fig. 1A). No lesions were observed in the animals sacrificed after the seventeenth day. A summary of the macroscopic findings at autopsy is presented in Table 2.

Of the latter group of kittens, eight animals (Group II) which gave no evidence of decreased normal activity or loss of appetite at any time following experimental infection, were observed for 35 to 40 days. These animals began to pass dysenteric stools loaded with trophozoites on the fourth day following experimental infection. Trophozoites decreased markedly on the eighth day and were absent from the fourteenth day onward. No pathology was evident at autopsy.

Since the study was not planned as one in histopathology, tissues of six kittens only were examined microscopically. Trophozoites of *E. histolytica* were observed in all tissue sections made of ulcerative lesions (Fig. 1, B).

Two types of sera were studied, one from kittens experimentally infected with *E. histolytica* and another from hyperimmunized animals. Twenty kittens were employed in the production of immune serum; of these 20 animals, 12 survived the immunization procedure and were bled 7 days following the last inoculation of antigen.

Neither complement-fixing antibodies nor increased gamma-globulin concentrations were observed during amebic infection in the kitten. Pooled sera from 8-week-old animals immunized with washed culture trophozoites of *E. histolytica* showed a moderate increase in gamma-globulin and gave positive complement fixation (Table 3). The total protein nitrogen was essentially unchanged following intestinal amebiasis (normal: 6.73 mg %; post-infection: 6.39 mg %); a slight increase was observed following active immunization (7.42 mg %).

DISCUSSION

The complexity of the physiology of the intestine, the inability to grow *E. histolytica* in the absence of microbial associates, and the intricacies of the psycho-physical response to stress, make it difficult at the present time to evaluate the host parasite relationship in amebiasis. Four factors appear to be significant in determining the progress of experimental infection in the kitten, these being: (1) nutrition and food intake, (2) bacterial flora of the intestinal tract, (3) changes in the in vivo environment occasioned by "stress state" of the animal and (4) intercurrent infection.

The importance of nutrition in relation to host resistance has been recognized as a major factor in modifying the virulence of many parasites, especially those that reside in the intestinal tract. Clinical studies have pointed out that acute amebiasis in human subjects is related to poorly balanced diet mediated through the changes induced in the intestinal flora (Elsdon-Dew, 1949, 1954). Similar observations have been made in studies on experimental amebiasis in the rat (Eskridge and Hegner, 1937).

In the present investigation it was observed that about 50 per cent of the kittens confined to small, separate cages and deprived of the oppor-

tunity for normal exercise or association with other animals, refused food entirely or stopped eating on the first and second post-operative days. Of this group 18 kittens developed amebic lesions. Litter mates assigned to Group II and maintained under environmental conditions more favorable to the animal seldom refused food before the fifth post-operative day and had a significantly lower incidence of infection (Table 2). These data suggest that in the kitten nutritional inadequacies resulting from refusal to eat may decrease the level of local tissue immunity which is a property of the intestinal mucosa and potentiate the tissue-invading phase of *E. histolytica*. Further study of the effect of undernutrition on infection with *E. histolytica* in the kitten seems indicated.

The marked effect of emotional states on the gastro-intestinal tract has been well known for years. Because these effects are usually transient, it is not always realized that they may be prolonged if the emotional state persists. Although it is difficult at the present time to appraise the significance of the "stress state" on every physiological process in the integrated animal, it is reasonable to assume that changes in the physiological condition of the host may modify the in vivo environment in such a manner as to alter the virulence of the parasite.

Kittens 8, 9, and 10, first generation half-Siamese and extremely irritable, nervous animals, were brought to the laboratory before they were weaned and were fed by bottle 1 week prior to self-feeding. During this time they developed diarrhea which persisted after they began to take solid food. Repeated stool examinations were negative for parasites. At the age of 5 weeks (280–300 gm) they were inoculated intracecally with *E. histolytica* and sacrificed at 28, 48, and 56-hour intervals when moribund. Although it has been observed that kittens sacrificed in less than 40 hours rarely develop intestinal lesions (Rees, 1929), kitten 9 developed one lesion approximately 0.2 cm in diameter in the region of the ileocecal valve 28 hours following experimental infection. Kitten 10, sacrificed 56 hours following experimental infection, developed fulminating amebiasis (Fig. 1, C and D). The significance of these infections is a fascinating matter for speculation.

There is at the present time no adequate explanation of the mechanism by which *E. histolytica* is able to invade the tissues of the host. Assuming that when fear is a dominant emotion the colon is kept in an almost constant state of hyperactivity, a possible relationship may be established between the "stress state" of the half-Siamese kittens and the persistent diarrhea observed in these animals.

It is highly probable that an altered intestinal emptying-time may be a factor in modifying the in vivo environment of an intestinal parasite. If these assumptions are valid, it may be postulated that invasiveness was induced in *E. histolytica* by the presence of substances which have not been recognized in vitro because they are elaborated by the ameba only in response to some factor(s) peculiar to a specific in vivo environment.

A second possibility that may be advanced to explain the enhanced invasiveness of the ameba in these three kittens is that some substance had been added to the inoculum giving the ameba more ready access to the tissues.

Recent studies using germ-free hosts (Phillips *et al.*, 1955) indicate that the intestinal flora is important in determining whether or not *E. histolytica* will become established in the intestine. Many of the organisms which support the ameba in vitro produce hyaluronidase as a product of their metabolism. These same species of bacteria may be found as normal inhabitants of the intestine. Assuming that *E. histolytica* makes use of a hyaluronidase-hyaluronic acid enzyme-substrate system as one of the possible modes of tissue invasion, it is tempting to speculate that if these bacteria were present in sufficiently large numbers in the intestines of kittens 8, 9, and 10, their metabolites may have enhanced the infectivity of the ameba resulting in the extensive ulceration evident at autopsy. Although these speculations are based on certain assumptions, it is possible that cytolysis may be a major method of invasion under one set of environmental conditions and a minor factor when a different in vivo environment obtains. Further studies should consider the relationship between the presence of psychological tension in the host and increased pathological activity of *E. histolytica*.

In parasitic infections in general an intercurrent infection either enhances or depresses the natural resistance of the host. If an animal is infected with one parasite at the time it is exposed to a second, there may be a greater chance for infection because of some depressing effect of the initial in-

fection on the resistance of the host. In man the findings of a disproportionately large number of infections with two or more species of amebae or with amebae and intestinal flagellates is suggestive that intercurrent infection may depress the natural resistance to secondary infection with *E. histolytica* (Boughton and Byrd, 1938).

Coccidiosis, a protozoan disease widely prevalent in the animal world, is a self-limiting infection not infrequently found in the cat. The disease is transmitted from animal to animal by the ingestion of ripe oocysts; symptoms generally subside shortly after the first appearance of the oocysts although the parasite may occur in the feces for several months after their initial appearance. Since 8 per cent of the kittens that were presumably parasite-free when brought to the laboratory developed coccidiosis during the course of the experiment, it is obvious that a 3 to 4 day observation period is inadequate to determine whether animals are parasite-free before they are experimentally infected with *E. histolytica*. Fortunately, these animals had been assigned to the Group I series and it was possible to check the spread of the parasite by sterilizing the contaminated cages. However, under other experimental conditions and/or with another group of animals, the incidence of infection with protozoa and helminths might be considerably higher than was observed in the present investigation. The development of amebic lesions in 5 of the 6 kittens (83%) infected with *Isospora* as compared with 49 per cent in the uninfected group indicates that in the kitten intercurrent infection depresses the natural resistance of the animal to secondary infection with *E. histolytica*.

The basic mechanisms operative in the immune response against an animal parasite are probably the same as those functioning against other infectious agents. Before an immune response occurs the host must be stimulated by the antigens of the parasite. This stimulation is dependent largely on the invasion of the host tissues by the parasite itself. Clinical and experimental evidence suggests that *E. histolytica* may reside in the lumen of the intestine of the host as a commensal organism feeding on bacteria and other material and forming cysts to complete its life cycle. At the present time we do not know either the circumstances which induce tissue invasion by the parasite or the extent of tissue damage necessary for the development of a demonstrable immune response.

Under the conditions of this experiment, no significant antigenic stimulation, as reflected in changes in the serum proteins, appears to follow purely intestinal infection with *E. histolytica* in the kitten (Table 3). Here the findings are in agreement with those of Comer *et al.*, (1956) who studied the serum proteins in dogs following experimental infection with ameba. The decrease in albumin and compensatory increase in alpha 2-globulin from the twelfth to the seventeenth post-infection day may be regarded as nonspecific aspects of the changes observed in the acute phase of many infectious diseases (Stauber, 1954).

As further evidence of the poor absorption of antigenic components of *E. histolytica* through the intestinal mucosa, serum antibodies (complement-fixing) could not be demonstrated when the ameba did not invade the tissues (Table 3, 35 to 40-day group), or when the invasion was superficial (5th, 10th, 12th, 15th, to 17-day groups). If antibodies were formed, their titer was too low to be determined with the antigen available. It is possible that resistance developed to intestinal infection with *E. histolytica* may be a local phenomenon involving changes in the cells of the mucous membrane of the intestinal tract which are not passed on to the blood stream.

On the other hand, when *E. histolytica* antigen was inoculated intraperitoneally, the antigenic components of the ameba were in a better position to penetrate into the somatic tissues of the host and to elicit a more vigorous antibody response. The appearance of complement-fixing antibodies and an increase in gamma-globulin in hyperimmunized kittens may be related to the increased number of antibody-forming sites exposed to antigenic stimulation.

SUMMARY

The present experimental results indicate strongly that acute amebiasis in the kitten is probably not the result of infection with the parasite *per se* as usually conceived. It is possible that in the kitten the severity of experimental infection with *E. histolytica* may be modified by variable characteristics of the individual animal, its nutritional status, the presence of intercurrent infection and the psycho-physical response to stress resulting in an overwhelming infection in a species of animal which under more normal con-

ditions may be only moderately susceptible to infection with the ameba.

ACKNOWLEDGMENT

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NECROTIC ARACHNIDISM

J. A. ATKINS,[1] C. W. WINGO,[2] W. A. SODEMAN[3] AND J. E. FLYNN[4]

In the United States reported cases of arachnidism (spider bite) are usually attributable to the black widow, *Latrodectus mactans*. In fact, all standard references list *Latrodectus* spp. as the only North American spider capable of inflicting serious injury to man. The venom of *Latrodectus* is neurotoxic with symptoms so striking there is little danger of confusion with those of other venomous animals. Authentic cases of arachnidism other than latrodectism are rare in the American literature. Gotten and MacGowan (1940) reported a case of blackwater fever resulting from the bite of a "large brown spider." Presumably the spider was not recovered but was thought to be *Amaurobius ferox*. Tinkham (1946) reported a case allegedly involving a "poison-squirting" spider, *Peucetia viridens*.

In the tropical regions of the world, particularly in South America, arachnidism is apparently much more common and several species have been identified that are injurious to man. Brazil and Vellard (1925) studied the venoms of *Ctenus ferus*, *Ctenus nigriventer*, *Trechona venosa*, *Nephila cruenta*, and *Lycosa raptoria*. Venom of *Ctenus* spp. affected the nervous system exclusively while that of *Nephila* and *Lycosa* was local in action. They reported the venom of *Lycosa raptoria* to be very potent and it was incriminated in necrotic arachnidism in São Paulo.

Macchiavello (1937) established the role of *Loxosceles laeta* in cutaneous arachnidism or "gangrenous spot" of Chile. In a later paper, Macchiavello (1947) reviewed the South American literature concerned with gangrenous spot and described a series of cases, all characterized by the appearance of an initial lesion caused by the bite of *L. laeta*. In these, irritation or burning was followed by erythema, edema and later the formation of a blister. The blister ruptured, the area became "gangrenous," the eschar sloughed and a superficial ulcer formed. He reported no symptoms other than localized pain, anesthesia of the necrotic area and hyperesthesia of the tissues in the immediate vicinity. The healing of necrotic tissues took place very slowly. In addition to these typical cases of localized necrosis caused by *L. laeta*, Macchiavello presented two cases in which the patients had not only cutaneous necrosis but also renal disturbances. This latter type was designated as viscero-cutaneous arachnidism by Macchiavello. The passing of black urine by these patients is suggestive of the American case history reported by Gotten and MacGowan (1940).

Atkins, Wingo and Sodeman (1957), in a preliminary report, suggested the possible role of *Loxosceles reclusus* as the causative agent of necrotic spider bite in Missouri and other midwestern states. As indicated in the preliminary report, most cases of necrotic spider bite may be erroneously interpreted as insect bite. Since the biting animal is usually crushed, or destroyed, positive identification of the agent is not possible.

The senior author, practicing in rural and urban Missouri over a period of years, has treated a number of patients for effects of the bite of a "brown spider." Although in all cases the patients have failed to capture and deliver the spider for identification, they invariably describe it as brown. This observation, along with the absence of the familiar symptom of latrodectism, precludes the possibility of black widow bite.

In the past three years in Central Missouri five patients in whom a clinical picture of the type termed necrotic arachnidism has developed have been observed and treated. In one case the patient had seen the spider inflicting the bite and was encouraged to collect spiders resembling it for identification. The only spider resembling her original description the patient was able to collect was a single female of *Loxosceles reclusus*. Some time later the same house was visited and *L. reclusus* found along the cellar steps.

A white male who received a sub-necrotizing dose of venom illustrates a circumstance in which the patient was bitten lightly and was unaware of it until a wheal formed and general discomfort was experienced. A survey of the premises of this

[1] Department of Pathology, School of Medicine, University of Missouri. Deceased Dec. 9, 1956.
[2] Department of Entomology, College of Agriculture, University of Missouri.
[3] Department of Medicine, Jefferson Medical College of Philadelphia.
[4] Department of Pathology, School of Medicine, University of Missouri.

patient revealed a general infestation of *L. reclusus* in outbuildings and a very heavy population in the entrance and along the steps of a storm-type fruit cellar which was visited daily by members of the household.

The following case report illustrates a striking example of the clinical picture observed.

T. W., a white female, age 33 years, was awakened at night by a stinging sensation in the left deltoid area. She had apparently turned on her left side in bed just before the stinging sensation occurred. After putting on the light she saw what she thought was a brown spider on the adjacent wall. However, she was more concerned with the pain at the time and did not connect her symptoms with the spider. Immediately after the stinging sensation there appeared a reddened area on the deltoid area. The pain became intense. There was also radiation down the arm when pressure was applied to the area surrounding the bite. Upon examination of the patient twelve hours after the bite occurred, the blood pressure in the right brachial artery was 126/82 mm. Hg, the oral temperature was 99.6° F., pulse was 96 beats per minute. Respirations were 22 per minute. The important physical findings concerned the area in the left deltoid region. There was a whitish, edematous, central area about 1.5 cm. in diameter with a wide irregular zone of inflammation about it (Fig. 1). The area was exquisitely tender. Within another 24 hours there was extensive extravasation of blood into the red zone surrounding the central white area (Fig. 2). A generalized scarlatiniform eruption appeared over the body and over both upper and lower extremities (Fig. 3). It remained three days and then gradually faded. The white blood count was 11,500 per cu. mm. with 73% neutrophils. The blood hemoglobin was 14 gm%. The urine contained only a trace of albumin. In succeeding days the central area of the bite became necrotic, turned black and finally sequestrated (Fig. 4). A deep ulceration extending down to the muscle appeared in a week. The edges were ragged but sharp and somewhat overhanging. Tenderness of the area disappeared gradually. Over a period of ten weeks healing and scarring occurred.

About 12 hours after the bite the patient was given prednisone, 15 mg. three times a day. This was continued for a week and then the dose was gradually reduced over the next week. No effect was noted.

The characteristic feature of the above patient is the marked area of necrosis at the site of the supposed bite. The picture differs remarkably from that of a black widow bite, in which necrosis is uncommon except as the result of excessive local treatment, use of tourniquets, incision into the bite, use of excessive suction, the application of corrosive chemicals, or from infection at the site. Severe pain developed locally at the site of the bite and within the next 24–36 hours a systemic response developed characterized by restlessness, fever, and a generalized scarlatiniform eruption. In the five instances we have seen, this latter eruption has been observed but once and this was in the above described patient. In each patient at the local site of the bite there developed an intensely ischemic area surrounded by redness and extravasated blood. The white area progressed to a rapid necrosis turning violaceous, then black and dry. In a few days to a week the dead tissue sloughed leaving a deep ulcer with sharp ragged edges and a yellowish white base. Upon washing out the exudate the underlying muscle tissue could usually be seen at the core of the ulcer.

In the acute stage, tenderness in the area was exquisite and touching the skin caused lancinating pain. Severe symptoms lasted five to seven days. Slow gradual evolution over several weeks led to complete healing with scarring. Fever developed, at times with an elevated white blood count which in one instance reached 25,553 per cu. mm. Protein appeared in the urine. We found no gross hemoglobinuria. No spectroscopic studies were done. MacKinnon and Witkind (1953) reported that hemoglobinuria with anemia has been observed. In our group there were no deaths. Again, in Latin American reports, deaths have occurred, especially in the very young and in the very old.

Therapy, in our hands, has been in large part supportive and symptomatic. Prednisone was utilized in the case described above but results were not dramatic. Others have used steroids without much effect.

The striking similarity of these cases of necrotic spider bite to cutaneous-arachnidism of Chile prompted experiments with *Loxosceles reclusus* and laboratory animals. *L. reclusus* in several stages were collected in the field and kept individually in pint-sized ice-cream cartons in which the center of the lid had been replaced with heavy cellophane. Feeding was accomplished twice

FIG. 1. This is a close-up of the left deltoid area of a 33-year-old white woman 12 hours after a brown spider bite. There is a zone of hemorrhage with a central, softened, elevated area where necrosis is occurring. (The elevated spots near the upper and lower margins of the photograph are nevi, of which the patient had many.)

weekly by dropping two or three nymphs of the English house cricket, *Acheta domestica*, through a hold in the top normally covered by Scotch tape. Water was made available at least once a week. The cartons, kept in a wire insect cage, were stored at 76–77° F. Both sexes of *L. reclusus* have been kept under these conditions for over eleven months.

Biting of laboratory animals was accomplished by confining spiders, first anesthetized by carbon

FIG. 2. The picture was taken 48 hours after the bite. By now the hemorrhage is more localized and the central area of softening is bigger. The epithelium is still intact but elevated by the underlying pus.

dioxide, under a plastic membrane taped over bare skin of the hip or belly. The membrane was taped loosely so that the spider, upon recovering from the anesthetic could move about freely. A few spiders bit spontaneously when irritated by slight pressure of the membrane. Those not responding to this stimulus were irritated by tapping the base of the chelicerae with forceps. In some cases it was necessary to irritate the spider and then present with forceps a fold of skin which was promptly pierced by the fangs. There was considerable individual difference in biting.

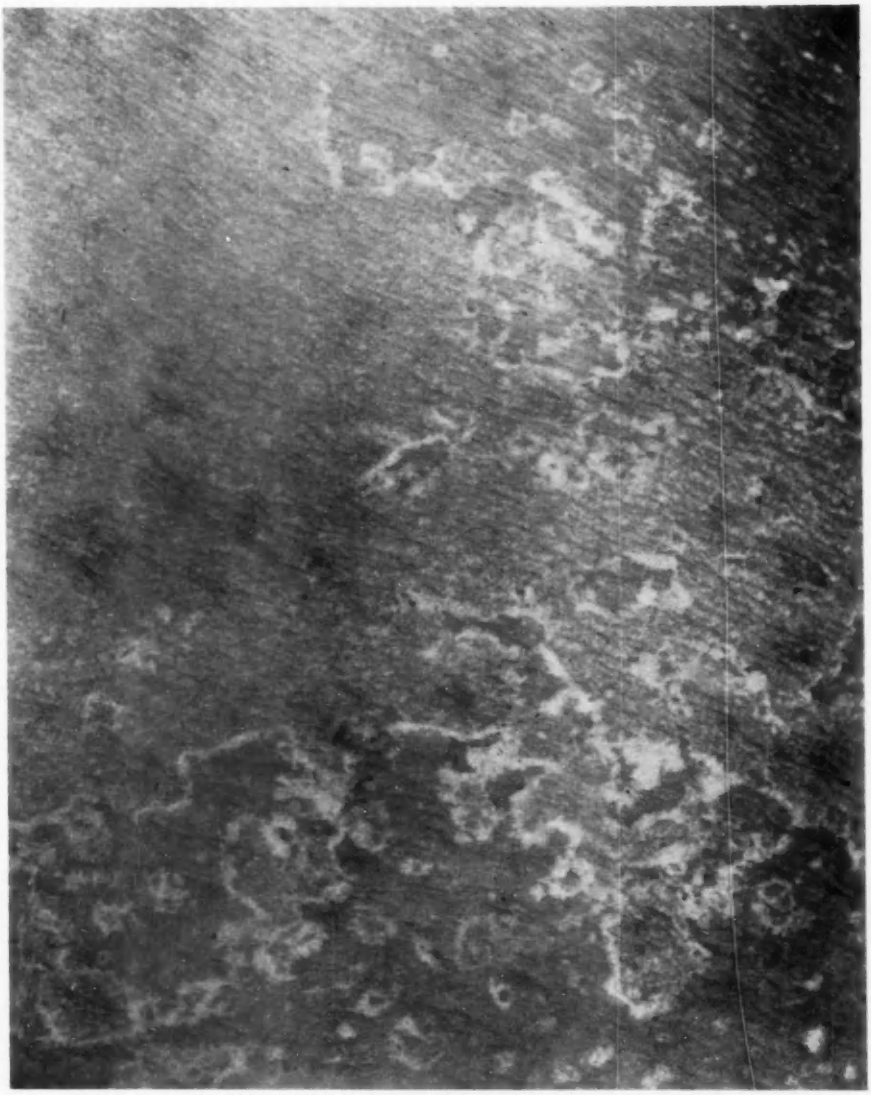

FIG. 3. This picture was taken 10 days after the spider bite. It is a close-up of the outer aspect of the thigh. Note the desquamation of the skin. This patient had a generalized scarletiniform eruption 24 hours after the bite, and desquamation started after two days, before this photograph was made.

Some bit and detached very quickly, others remained with the fangs sunk into the skin for from five to ten seconds and in some extreme cases held to the skin until forcibly detached. Almost without exception, the amount of venom injected, as evidenced by the effect on the laboratory animal, was proportional to the length of time the fangs remained in the skin.

Four domestic, cross-bred adult rabbits weighing from 7–8 pounds were used in the pre-

FIG. 4. This is a close-up of the bite. The necrotic tissue has sloughed leaving an ulcer filled with pus. When the ulcer was washed with saline the base could be seen to extend down through the subcutaneous tissue to the deltoid muscle.

liminary experiments. The first rabbit, bitten three times by a large adult female *L. reclusus*, developed wheals 10–15 mm. in diameter and died violently in less than 13 hours. Post mortem examination revealed skin at site of bites 8 mm. thick due to hemorrhage and edema; profuse subcutaneous hemorrhage and edema over the shoulder area to toes; petechial and ecchymotic hemorrhages in most of the large skeletal muscles, serosa of stomach, small intestine and thymus;

shallow erosions up to 3 mm. in diameter in mucosa of the stomach. An incidental finding was intussception in the anterior portion of the jejunum and the distal end of the colon.

A second rabbit received a single bite on the hip by a small female spider. The site of the bite was red and congested within 5 hours. A wheal appeared within 7 hours which increased in size and thickness for the next 24 hours. During the second day the rabbit was lethargic, the bitten leg was held stiff and weight-bearing avoided. A large amount of yellow liquid fecal matter was passed. By the third day the subject had lost 7 1/2 ounces of body weight, the wheal was stabilized with the edges thickening and a secondary pocket was forming below the initial area around the bite. The rabbit was more active on the fourth day, eating and drinking normally. By the fifth day muscular action of the affected leg appeared normal, the feces were normal solid pellets and the skin at the site of the bite was turning bluish-purple with the edges of the wheal red and thickened. On successive days there were no general changes and the wheal blackened at the center and the surface of the affected skin dried, becoming hard and parchment-like. On the tenth day this dry, black area sloughed. Sloughing continued and was complete by the fifteenth day. At this time the area involved was about 30 mm. by 60 mm. The edges of the wound were raised, thickened and red with the limit between healthy and affected tissue sharply defined. Healing of the area started within a short time and was complete 35 days after the rabbit was bitten. The subject had lost 16 per cent of original body weight during the period.

In subsequent experiments involving six rabbits either bitten once by *L. reclusus* or injected intradermally with small amounts of venom, death occurred in two cases, and the formation of an eschar, necrotic area and sloughing in the other four cases. Considerable variation in the size of the necrotic area was observed. The largest ulcer measured 30 × 60 mm. while the smallest affected area sloughed only at the small area marked by the chelicerae of the spider. In two cases the rabbits had intense diarrhea on the second day following the bite.

As rabbits seemed quite sensitive to the bite of *L. reclusus* and have a rather thin skin, guinea pigs were used as laboratory subjects in the next series of experiments. A total of 20 guinea pigs were bitten by *L. reclusus*. All showed the typical initial hemorrhagic area around the bite followed by formation of a wheal. Eleven of the bitten subjects died within 12 to 70 hours. General systemic disturbance was evident among these animals before death and in one case bloody urine was discharged. The remaining 9 guinea pigs developed the usual black eschar which sloughed and eventually healed. The size of the affected areas varied from pinpoint lesions to necrotic areas 15 × 30 mm. in size.

In another series of experiments adult guinea pigs were killed at varying intervals after they were bitten by *L. reclusus*. In all cases complete autopsies were done. Sections were taken of the skin at the site of the bite, and also of the heart, lungs, kidneys, intestine, liver, spleen and reproductive organs. The significant changes were limited to the site of the bite. The viscera, including the kidneys, were normal, but in this group of animals none had gross hematuria. Some of the animals were pregnant. Gestation appeared to be unaffected as far as gross autopsy findings were concerned. The following four protocols illustrate the histological changes that occur in experimental necrotic arachnidism.

Guinea pig 564-X107 was sacrificed two hours after the bite (Fig. 5). The most conspicuous change was hemorrhage in the dermis, subcutaneous tissue and superficial muscular layer (Fig. 6). The capillaries within the hemorrhagic zone were markedly dilated and filled with red blood cells. In some vessels the red blood cells were fused to form hyalinized masses. At the margin of the hemorrhagic zone there was a slight leukocytic infiltration.

Guinea pig 511-X10: This animal was sacrificed 24 hours after the bite. The histological changes were most marked in the epidermis, dermis, subcutaneous tissue and superficial striated muscle. The hair follicles at the site of the bite were disintegrating, and there was extensive necrosis of the epidermis (Fig. 7). There was a marked leukocytic infiltration about the hemorrhagic zone, and many of the capillaries contained small, hyalinized thrombi (Figs. 8 and 9).

Guinea pig 536-X112: This animal was sacrificed three days after the bite (Fig. 10). The skin was ulcerated where the necrotic tissue sloughed (Fig. 11). There was a zone of coagulation necrosis extending into the subcutaneous fat. Deep within the subcutaneous tissue there was a

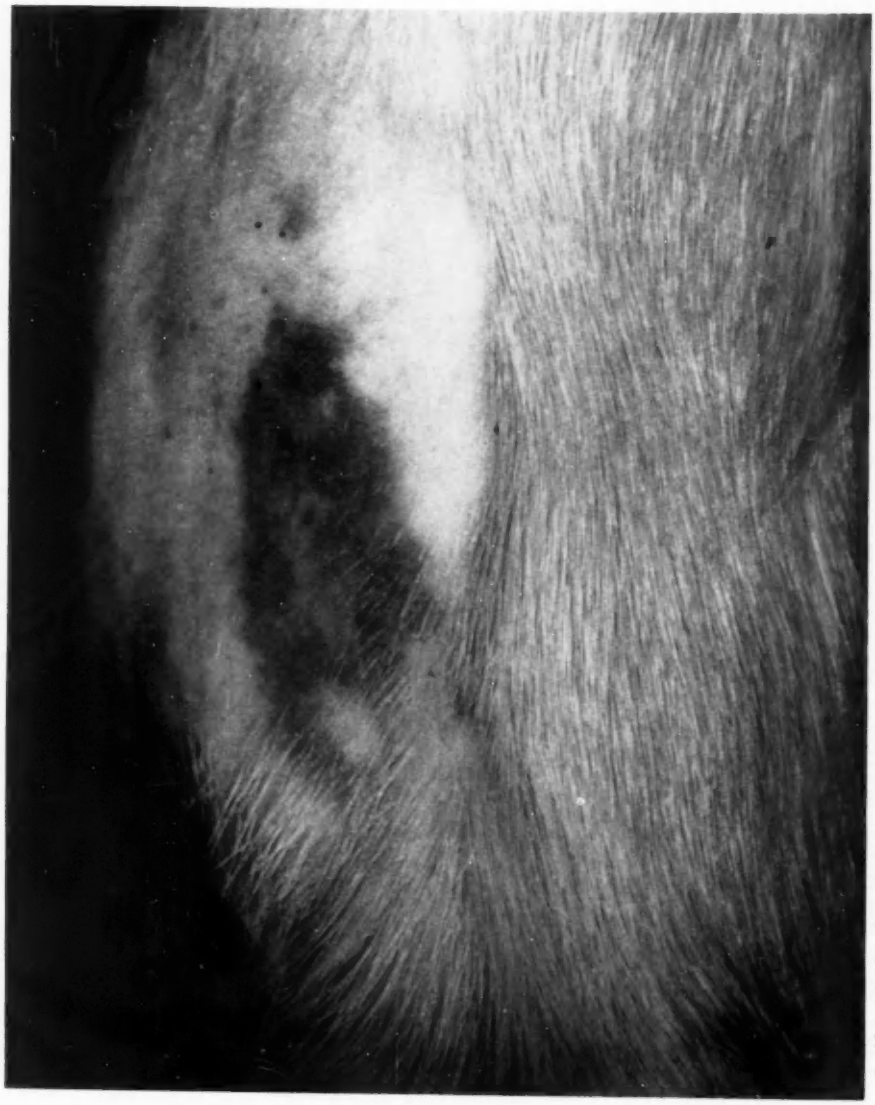

FIG. 5. Above is the shaved abdomen of a guinea pig 2 hours after a brown spider bite. Note the extensive area of hemorrhage.

FIG. 6. This is a photomicrograph of the subcutaneous tissue and underlying muscle at the site of the bite. It shows the changes present two hours after the bite occurred. In the lower one-third of the photograph is striated muscle. In the upper two-thirds is subcutaneous tissue. Note the marked hemorrhage of the subcutaneous tissue. Near the center of the photograph is a dilated blood vessel, the wall of which is probably necrotic or severely damaged. This accounts for the extravasation of red blood cells. 120 ×

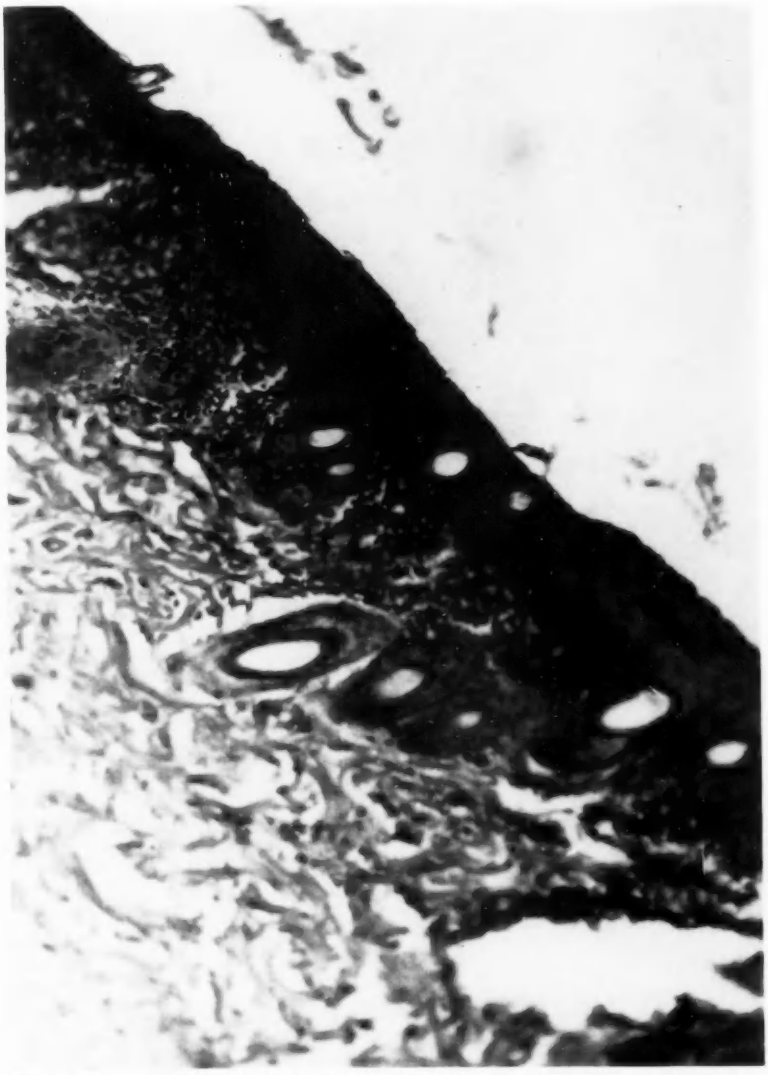

FIG. 7. Twenty-four hours after the bite. Passing obliquely across the photograph is the outer aspect of the skin. Note the marked necrosis of the dermis and epidermis. 120 ×

large abscess extending into the muscularis (Fig. 12). The abscess was surrounded by dense leukocytic infiltrations which, at the extreme periphery, gave way to beginning fibroblastic replacement (Fig. 13).

Guinea pig 558-X109: This animal was sacrificed 13 days after the bite. The abscess by this time had ruptured to the surface of the skin. Most of the abscess was filled in with scar tissue (Fig. 14). The connective tissue was quite cellular and moderately vascular. There was very little intervening collagen as yet between the cells (Fig. 15).

Almost all human cases of necrotic spider bite

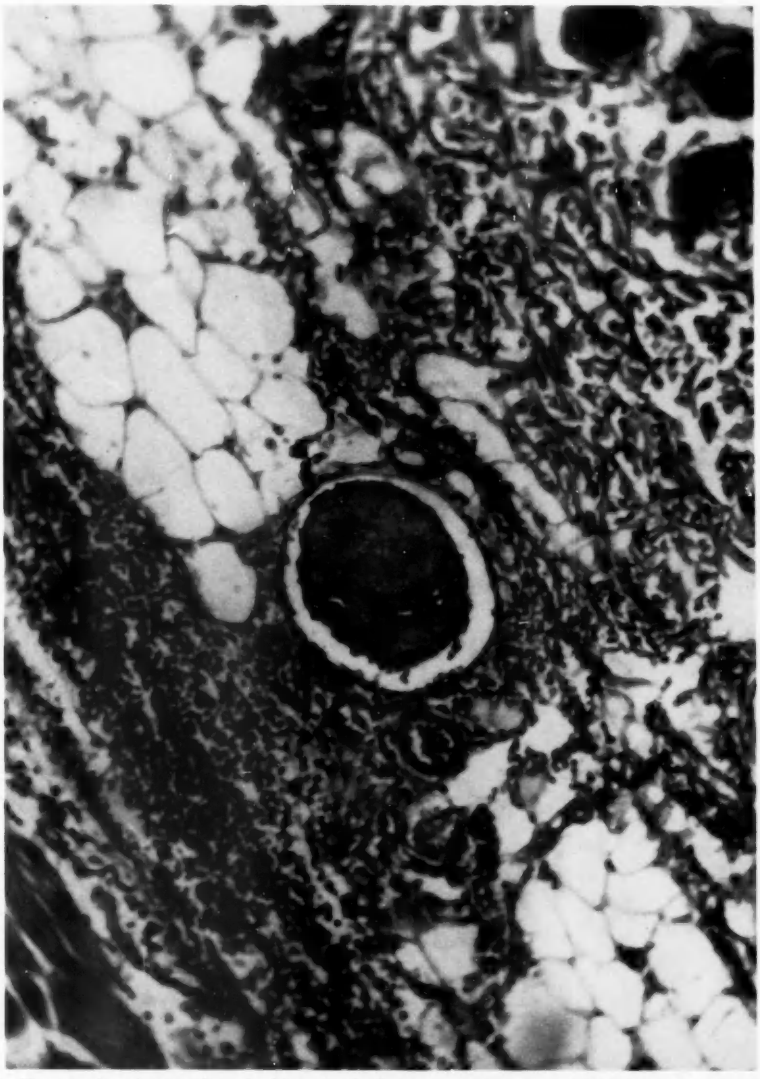

FIG. 8. Twenty-four hours after the bite. At the lower corner on the reader's left are a few strands of striated muscle. At the upper corner on the reader's right are a few hair follicles. Between is subcutaneous tissue. Note the marked hemorrhage and a beginning leukocytic infiltration. In the center of the photograph is a vessel containing hyalinized material. 120 ×

have been noted to occur when the spider was imprisoned within clothing recently donned or when the spider fell into bed clothing or open necks and sleeves of clothing. Presumably the person bitten crushed the spider by a sharp blow and then attempted to remove it. During the course of experiments described above with laboratory animals there were some cases in which the spiders beneath the membrane were inadvertently injured by forceps. It was noted

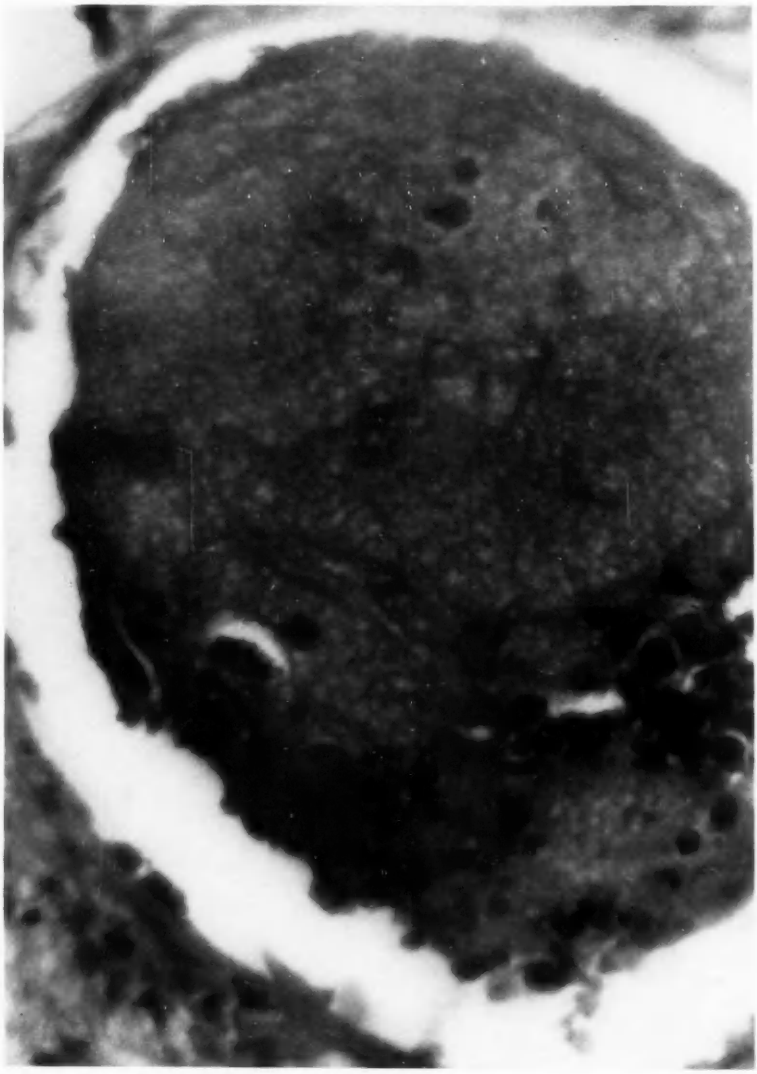

FIG. 9. Twenty-four hours after the bite. This is a high magnification of the occluded capillary seen in Fig. 10. Note that most of the lumen is occupied by an amorphous material. This probably represents masses of fused red blood cells. The necrotoxin of the spider venom causes necrosis of all tissues it contacts, including blood vessels, lymphatics and nerves. 460 ×

that although the spiders were severely injured and died within a matter of minutes they were capable of biting and producing necrosis or even death immediately after injury.

In order to simulate the human cases where the subject was bitten by a spider imprisoned within clothing, a series of experiments was run with specimens of *L. reclusus* imprisoned on the bare bellies of guinea pigs beneath gauze bandages. As soon as the spider recovered from the carbon

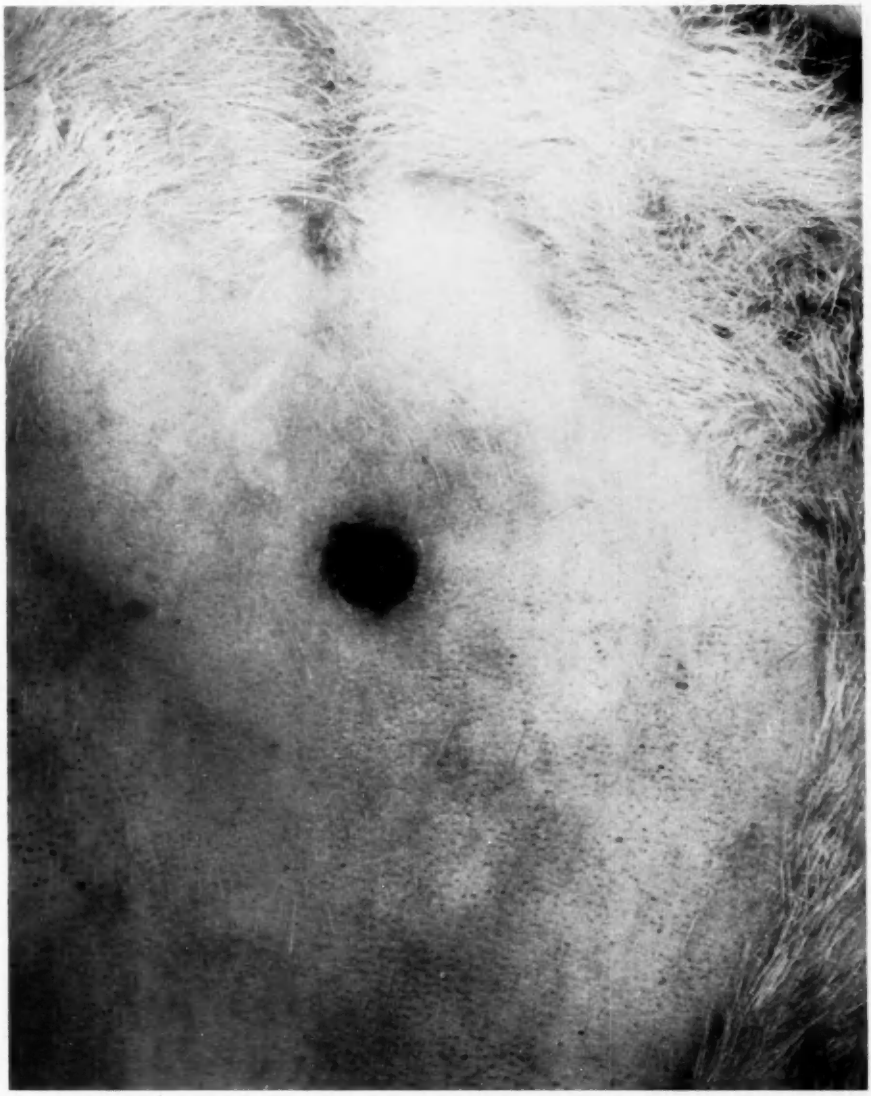

FIG. 10. This is the ventral aspect of the abdomen of guinea pig 3 days after a brown spider bite. In the center is an ulcer covered by a crust of fibrin and old blood.

dioxide anesthetic and was active beneath the bandage it was struck a sharp blow or series of blows. It was then held under the bandage for from five to fifteen seconds approximating the time one would require to remove a pest from the clothing.

In these experiments 16 guinea pigs were used. Positive bites followed by the production of typical necrotic lesions occurred in 6 cases. The remaining subjects were not bitten. It is evident from these experiments that in at least 30 per cent of the instances in which specimens of *L.*

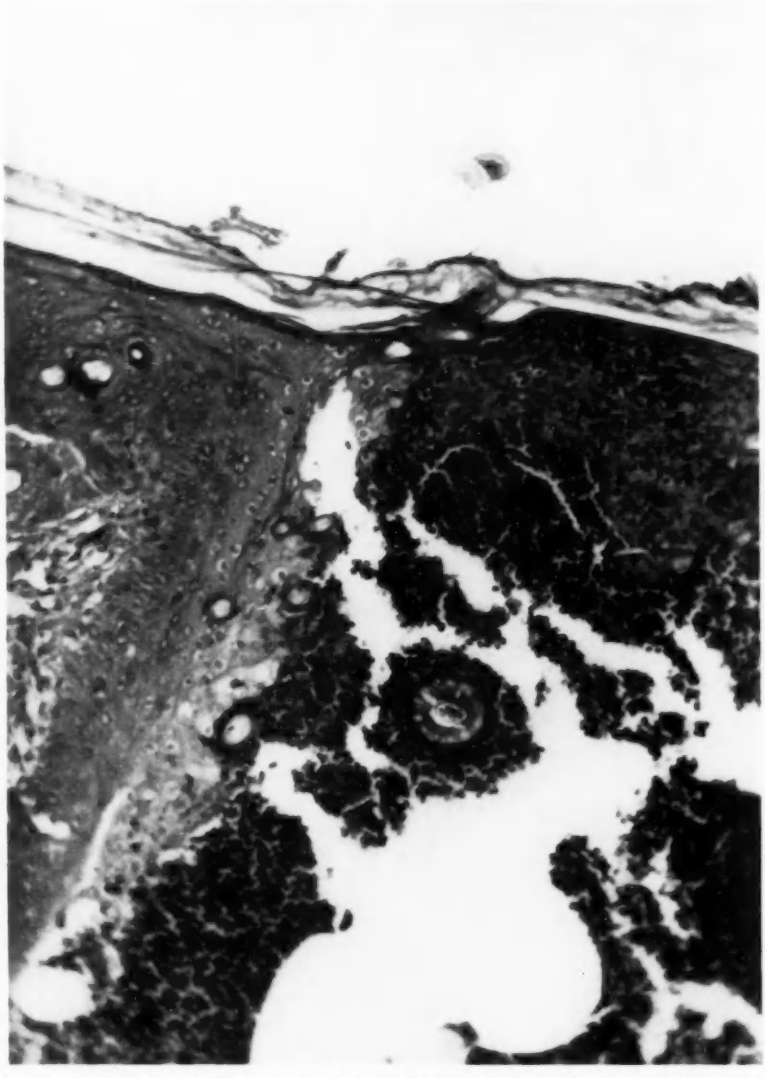

FIG. 11. Three days after the bite. On the right side of the photograph is pus which has almost ruptured through the overlying necrotic epidermis. 120 ×

reclusus became entangled between clothing and bare skin one could expect biting and the production of a necrotic spot to occur.

MacKinnon and Witkind (1953) carried out immunological studies to determine the specificity of the venom of the South American species *L. laeta*. They found rabbits to be immunized after 6–14 bites, and mice were protected by the serum of these rabbits. In the present studies two rabbits were immunized after four bites or injections of venom. One, a large female, after being bitten or injected seven times tolerated the injection of 2.5 glands of *L. reclusus*, macerated in normal saline, into the large vein of the ear. A control rabbit

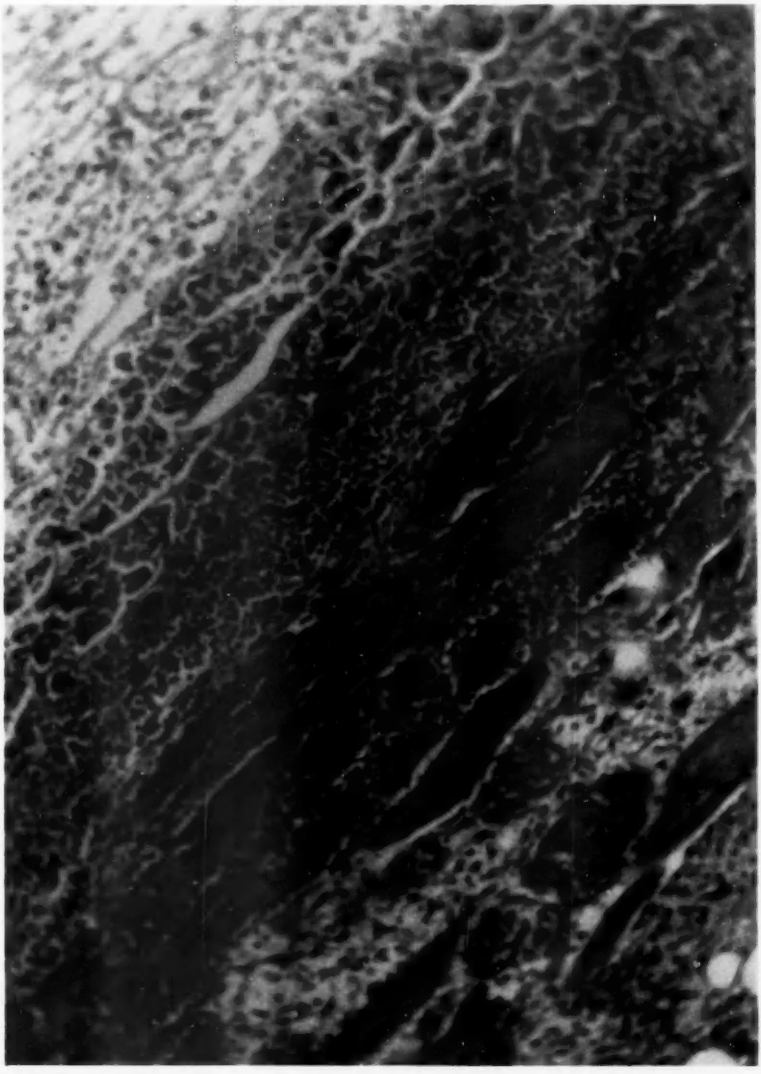

FIG. 12. Three days after the bite. In the lower corner on the reader's right are striated muscle fibers. At the upper corner on reader's left is the abscess. Between is a heavy leukocytic infiltration or "pyogenic membrane." 120 ×

receiving the same dose via the same route died violently within 21 hours. This preliminary test suggests further studies involving passive immunity which might be the basis for specific therapy by the use of serum of immunized animals.

Some confusion exists in the South American literature concerning the potency of venom from male and female specimens of *L. laeta*. Macchiavello (1947) reported a severe case (Case No. 27) of viscero-cutaneous arachnidism caused by a male specimen of *L. laeta*. Subsequent

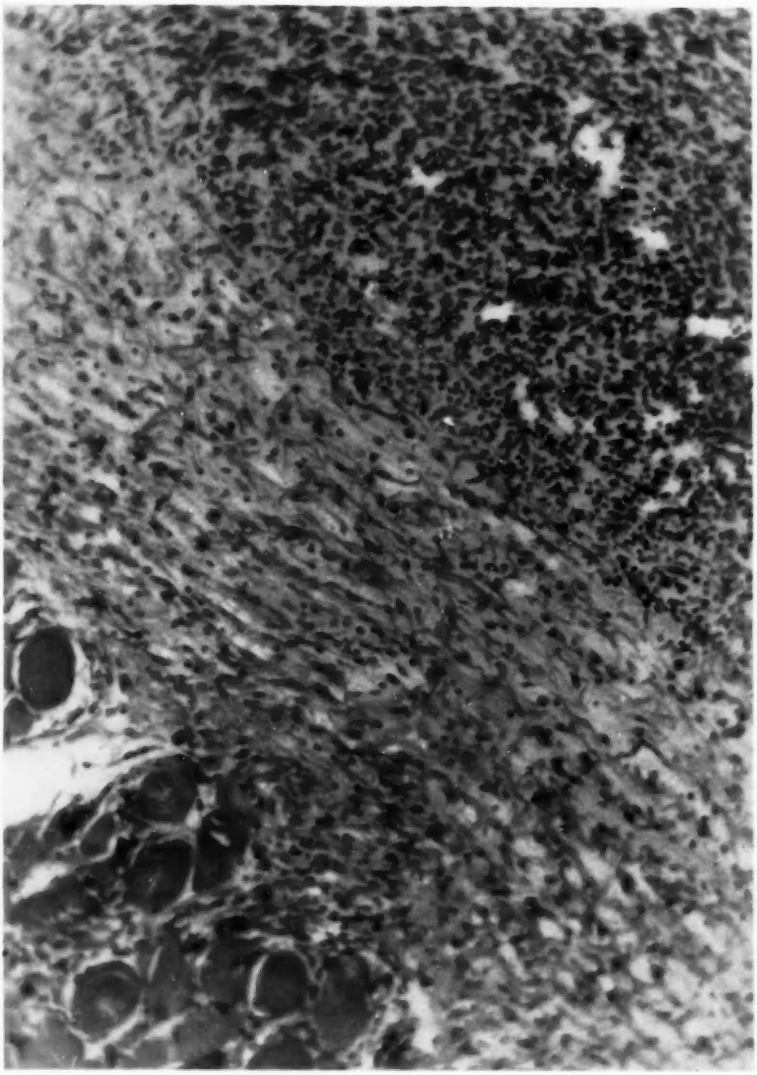

FIG. 13. Three days after the bite. At the lower corner on the reader's left there are a few striated muscle fibers cut on cross section. At the upper corner on the reader's right is part of the leukocytic infiltration that surrounded the abscess. Between is early fibroblastic proliferation. 120 ×

laboratory examination of the dried venom of this same specimen established its necrotizing action. However, MacKinnon and Witkind (1953) found no effect on mice either from bites of male spiders or from injection of assayed doses of male venom. The venom of male specimens of *L. reclusus* has been found to be as toxic as that of the female. The bites of male specimens produced necrotic lesions of various sizes both in rabbit and guinea pigs and death in the case of a number of the latter.

Loxosceles reclusus Gertsch and Mulaik (1940)

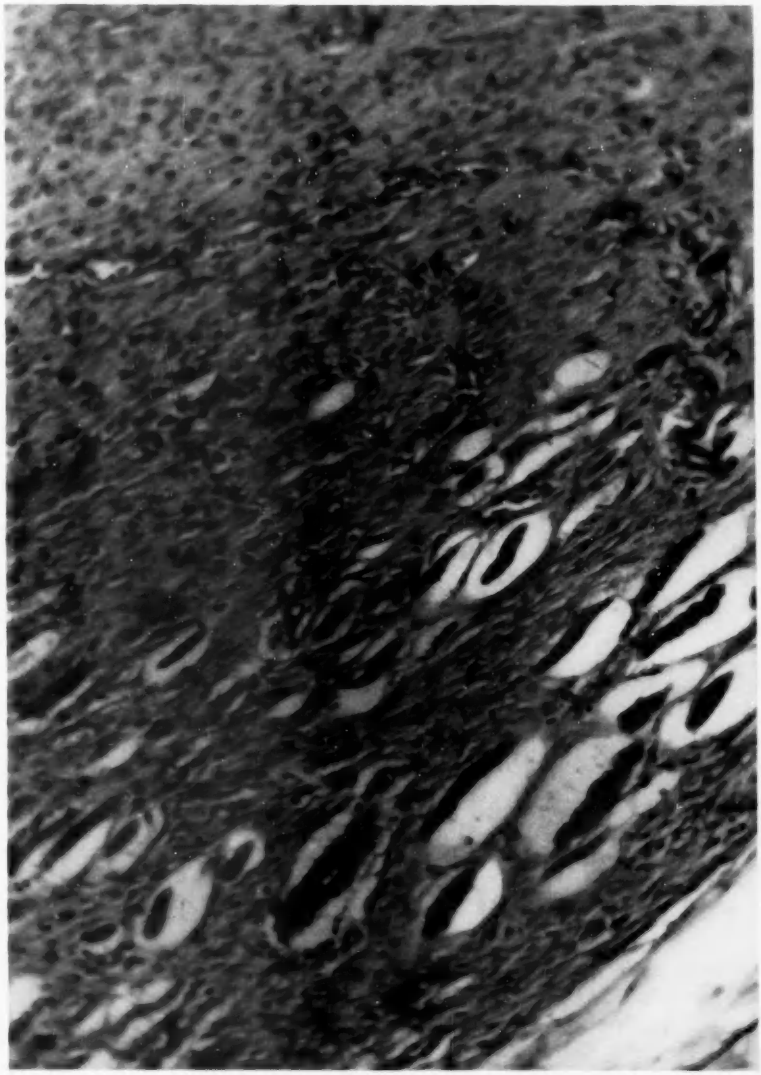

FIG. 14. Thirteen days after the bite. At the lower corner on the reader's right are strands of striated muscle fibers. Above is dense scar tissue filling in the abscess cavity. 120 ×

was described from Texas. The family Loxoscelidae, to which the species belongs, is commonly known as the false hackled band spinners. The species is of medium size, yellow or brown, with short, slight pubescence. The females (Fig. 16) are slightly larger, measuring 9 mm. total length, while the males (Fig. 17) measure 8 mm. The color range of specimens collected in Central Missouri is quite wide, running from dark brown, almost black, individuals to pale fawn or orange yellow. The cephalothorax of all specimens seen bears a median line of darker color extending

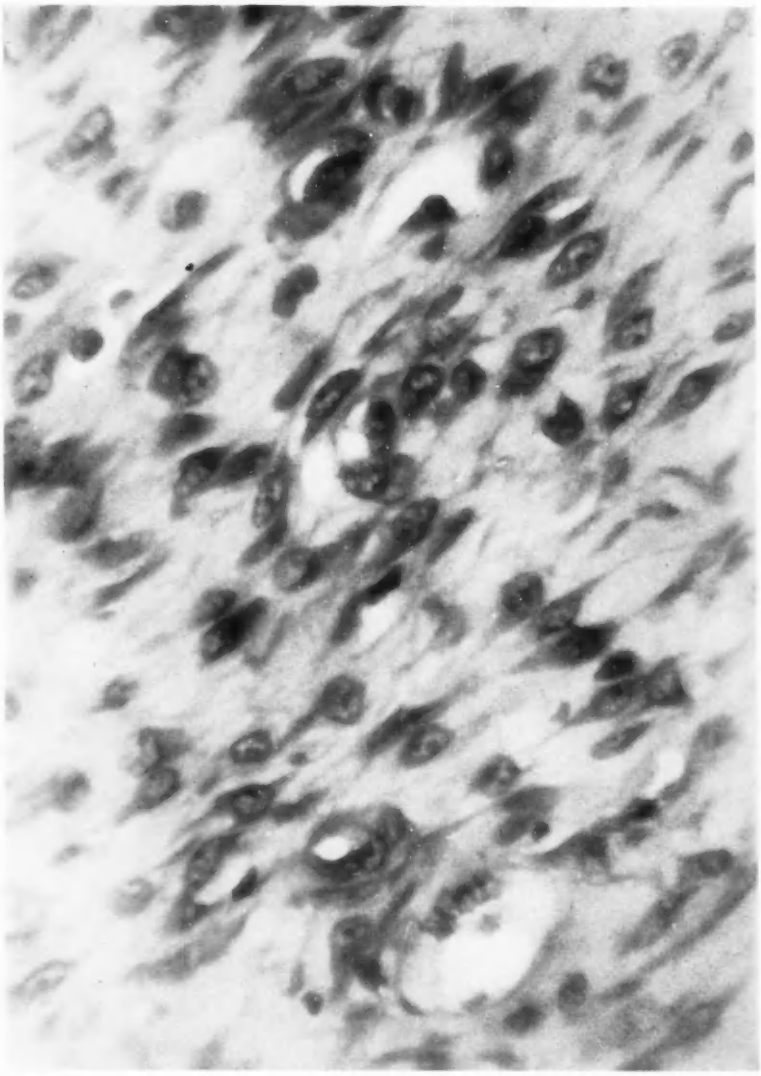

FIG. 15. Thirteen days after the bite: This is a high power magnification of the scar tissue. Note the proliferating fibroblasts with only a small amount of intervening collagen. In a few weeks more the cellularity would be greatly reduced and a more mature scar would remain. 460 ×

from the eyes toward the posterior margin. Proximally the legs are the same color as the body, but darken distally.

Specimens of *L. reclusus* captured in or near human habitations are usually found in outbuildings, closets or cellars which are cleaned infrequently and the contents of which are left undisturbed for relatively long periods. In such buildings they prefer to spin their irregular webs near cracks in foundations and steps, in boxes, or folds of clothing hanging from the walls. In common with most spiders *L. reclusus* shows no

FIG. 16. *Loxosceles reclusus* Gertsch and Mulaik female

FIG. 17. *Loxosceles reclusus* Gertsch and Mulaik male

aggressive qualities but escapes when slightly disturbed. Comstock (1940) says that *Loxosceles* are usually found under bark of trees and under stones on the ground. In Missouri *L. reclusus* appears to be a common house spider. The species is collected more readily in and around human habitation than in the field. According to Gertsch (1956, personal communication) a closely allied species, *Loxosceles marylandicus*, has been taken in St. Louis County, Missouri.

SUMMARY

Necrotic arachnidism in Missouri is strikingly similar to "gangrenous spot" or cutaneous arachnidism of South America caused by *Loxosceles laeta*. A typical case report presents a distinctive clinical picture quite unlike that due to a black widow bite. Severe pain develops at the site of the bite and within the next 24 to 36 hours a systemic response is likely to occur, characterized by restlessness, fever, and at times the development of a generalized scarlatiniform eruption. Locally an ischemic area develops, surrounded by redness and extravasated blood, and becoming rapidly necrotic, with a central sloughing area which forms a deep ulcer exposing at times the underlying muscles. Slow gradual evolution with scarring follows. Experiments with *Loxosceles reclusus*, the probable agent of necrotic arachnidism in Missouri, and laboratory animals showed that the spider produces a powerful necrotoxin which it can readily inject into the skin of mammals, producing in the guinea pig tissue damage characterized by local hemorrhage, acute inflammatory cell reaction and, within three or four days, liquefaction with the formation of an abscess resulting in granulation and scarring. There is some species variation, rabbits being more susceptible than guinea pigs. The venom of both sexes of *L. reclusus* was found to be toxic. Rabbits were immunized after four bites or injections of venom. *L. reclusus* has been collected readily from the homes of patients with "brown spider bite" and appears to be a common house spider in Missouri.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Dr. Loren D. Kintner, Department of Veterinary Pathology, for necropsy reports on rabbits used in the experiments; to Stirling Kyd, Extension Entomologist, for photographs of the spiders and to Dr. W. J. Gertsch, American Museum of Natural History, for examination and identification of spiders from the series used in the experiments.

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THE EFFECT OF CORTISONE AND PREDNISONE ON BULLOUS REACTIONS FOLLOWING TREATMENT OF FILARIASIS WITH DIETHYLCARBAMAZINE[1]

HARRY N. MARCH AND JACQUES LAIGRET

Institut de Recherches Médicales de l'Oceanie Francaise, Papeete, Tahiti and Department of Infectious Diseases, School of Medicine, University of California, Los Angeles

Diethylcarbamazine (Hetrazan or Notezine) is widely used in the Society Islands in a program designed to control Bancroftian filariasis in that area (see Kessel *et al.*, 1953). A dose of 6 mgm. per kilogram of body weight, administered once a month, is employed at the present time. This dosage is given to all persons in the population regardless of whether or not they are known to have a filarial infection. While in general this dosage is not accompanied by undesirable side-effects, unpleasant and sometimes serious reactions are seen in a small proportion of treated persons. Such reactions occur primarily in persons known to be infected with *Wuchereria bancrofti* who have microfilariae in their blood. Reactions are never seen when the drug is given to persons known not to be infected. They occur in their most severe form following administration of the first dose of diethylcarbamazine; subsequent doses fail to elicit a reaction, or the reaction is less severe.

Since diethylcarbamazine therapy results in sudden destruction of the circulating microfilariae, it is thought that reactions following its use are of an allergic nature, possibly analogous to a Herxheimer reaction in syphilis. One of the more severe and painful reactions following diethylcarbamazine treatment is a bullous eruption involving one or more limbs. This type of reaction is seen in approximately one per cent of treated cases. It appears in from 3 to 30 days after primary treatment, and is both painful and incapacitating. The affected limb becomes markedly edematous, with edema involving not only the area of eruption but the entire limb including the hand or foot. The patient develops fever, and complains of headache and generalized malaise. The bullae may vary in size and as the condition progresses tend to coalesce. Filled with fluid under pressure, the bullae may rupture spontaneously or because of trauma. If they remain intact, the overlying epidermis becomes necrotic and desquamates. In either case, secondary bacterial infection is very likely to result. Treatment in the past has consisted of antihistaminics, antibiotics, wet antiseptic dressings, and aspirin to relieve the pain. Antihistaminics have not proved to be of great value in modifying the course of the reaction, and the minimum time for recovery has been in the neighborhood of two weeks. Many cases run a more protracted course because of the severity of the secondary infection.

Adrenocorticotropin (ACTH) and the corticosteroids have been used to modify allergic reactions in various filarial diseases. McFadzean (1953) treated elephantiasis with ACTH, but without great success. Markell and Kerrest (1955) found cortisone effective in the treatment of that condition. Thompson (1956) used ACTH to relieve the acutely painful Calabar swellings which appeared during the course of diethylcarbamazine treatment of a patient with loiasis. Markell and Turner (1957) found cortisone and prednisone of great value in the relief of various allergic reactions, including those of a bullous type, occurring after diethylcarbamazine treatment in onchocerciasis. It was accordingly decided to try the effect of the latter two drugs on bullous reactions seen after diethylcarbamazine treatment of patients having filariasis.

TREATMENT

Following initial diethylcarbamazine treatment of a group of individuals on the island of Bora Bora, eight persons exhibited typical bullous reactions. The shortest period which had elapsed between administration of the drug and the onset of symptoms was three days, the longest period was twenty-seven days. The cases were first seen when symptoms had existed for an average time of five days; the earliest was seen on the first day, the latest on the fourteenth day after the start of the bullous reaction.

Four patients had bullous lesions involving one complete arm and shoulder, or forearm and hand.

[1] Aided by grants from U. S. Public Health Service—E-392—and The China Medical Board Inc., of New York.

TABLE 1

Results of treatment with prednisone and cortisone

Case no.	Age & sex	Days between administration of diethylcarbamazine and appearance of the bullous reaction	Duration in days of the reaction before treatment commenced	Total medication	Days to full recovery after treatment began
1	52, male	16	14	50 mgm. prednisone	2
2	45, female	15	5	100 mgm. prednisone	2
3	45, male	3	5	50 mgm. cortisone, 60 mgm. prednisone	3
4	45, female	4	4	50 mgm. cortisone, 60 mgm. prednisone	3
5	48, male	5	3	25 mgm. cortisone, 30 mgm. prednisone	1
6	14, female	9	1	50 mgm. cortisone, 30 mgm. prednisone	2
7	26, male	6	4	50 mgm. cortisone, 60 mgm. prednisone	2
8	30, male	27	3	35 mgm. cortisone, 80 mgm. prednisone	3

One had lesions covering both forearms, another both arms and forearms; another patient had involvement of one thigh, and the remaining individual had lesions over one leg and foot. Six patients were treated with a combination of cortisone and prednisone[2] and two with prednisone only. In the combined treatment, 25 mgm. of cortisone acetate was given by intramuscular injection on alternate days, and 15 mgm. of prednisone was administered in divided doses daily by mouth. The two patients who received prednisone alone were given the drug at the rate of 30 mgm. daily in divided doses for 2 days. In case No. 2 this was reduced to 20 mg. daily for two additional days. The total dose varied slightly in some of the patients, and is given in Table 1.

All patients reported freedom from pain and a definite reduction in swelling of the affected areas within twenty-four hours after therapy was begun. By the end of forty-eight hours' treatment, there was complete cessation of symptoms in every case with total absence of edema, and collapse and drying of the bullous lesions. No treatment other than the corticosteroids had been given to any of these patients.

In two cases treatment was administered in spite of the presence of minor secondary infection. No untoward results were seen and the infectious process was apparently unaffected by the administration of these quantities of corticosteroid drugs. It must be emphasized that corticosteroids must be given with extreme caution in the presence of secondary bacterial infection. Such infections, if severe, should be treated with antibiotics.

[2] Prednisone (Meticorten) used in these studies was supplied through the courtesy of The Schering Corporation, Bloomfield, New Jersey.

CONCLUSION

Cortisone and prednisone appear to be capable of arresting the bullous reactions sometimes seen after administration of diethylcarbamazine to persons having filariasis. It is probable that other corticosteroids would have the same effect.

RÉSUMÉ

L'emploi du diethylcarbamazine contre la filariose entraine chez environ 1 pour cent des Polynésiens traités, un type particulier de réaction appellé réaction bulleuse. Il s'agit probablement d'une reaction allergique, comparable à celle d'Herxheimer dans la syphilis. Elle est différente de la réaction plus fréquente attribuée à la destruction immédiate des microfilaires dans le courant sanguin. Elle survient plus tardivement. La cortisone et la prednisone semblent susceptibles d'interrompre ce type de réaction qui suit l'administration de diethylcarbamazine chez les personnes hébergeant des microfilaires.

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ISOLATION AND IDENTIFICATION OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS FROM MOSQUITOES BY TISSUE CULTURE METHODS*

HARTWELL H. WELSH, BEVERLY JEAN NEFF, AND EDWIN H. LENNETTE

Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley 4

As part of the continuing epidemiologic study of the arthropod-borne viral encephalitides in California, large numbers of mosquitoes (*Culex tarsalis*) are examined each year to obtain information on the prevalence of Western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) virus infections in this arthropod. Mosquitoes collected during these surveillance studies are tested for the presence of virus by the usual methods, viz., sub-inoculation into adult mice and embryonated hens' eggs. The size of the mosquito surveillance program necessitates the use of large numbers of animals and eggs, and the time and effort involved in the observations and manipulations associated with the use of these species are considerable. Current tissue culture methods because of their relative simplicity seemed to afford a possible means of expediting the isolation and identification of viral agents from mosquitoes. An inquiry was made into the feasibility of using such a method for this purpose and the findings are described in this communication. This report is concerned solely with the isolation of Western equine encephalomyelitis virus since, during the 1956 study season, only two isolations of St. Louis encephalitis virus were made in mosquitoes, which precludes at this time any evaluation of the method for the isolation of this virus.

MATERIAL AND METHODS

Collection of mosquitoes. Mosquitoes were collected from several areas in the San Joaquin and Sacramento Valleys, as previously described[1], for the purposes of mosquito surveillance studies (Lennette *et al.*, 1956). The collecting stations within each area were visited weekly to collect the captured mosquitoes. The predominant mosquito in the collections was *Culex tarsalis*, since the studies were concerned primarily with this species and the collecting stations were accordingly located in the preferred habitats of this species. The specimens in each collection lot were sorted according to species and pools of 50 female *C. tarsalis* were prepared, deviations from this number occurring only at the beginning and end of the season when the population densities were low, or in collecting areas where the prevalence of *C. tarsalis* was low. Each pool of mosquitoes was placed into a Pyrex tube which was then flame-sealed and stored on dry ice pending shipment to the laboratory. Specimens from all collecting stations within an area were held on dry ice in the area headquarters, and shipments were made from the field headquarters to the laboratory once weekly. In the laboratory, the specimens were tested immediately on receipt or within a few days thereafter.

Processing of mosquito pools. Each pool of mosquitoes was triturated in a mortar and suspended in a diluent consisting of 30 per cent normal rabbit serum in phosphate-buffered saline solution, pH 7.4. (The normal rabbit serum, pretested to rule out the presence of antibodies to the WEE and SLE viruses, was inactivated at 56°C. for 30 minutes prior to use. The buffered saline solution had the following composition: NaCl, 3.4 gm.; KH_2PO_4, 2.5 gm.; $Na_2HPO_4 \cdot 12H_2O$, 28.2 gm.; and distilled water to make 1,000 ml. Just before use, sufficient penicillin and streptomycin was added to the serum-saline to give a final concentration of 500 units of each per 1 ml.) The mosquito suspension

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We are indebted to Miss Mary Martins for valuable technical assistance.

[1] The field aspects of the mosquito surveillance studies are under the direction of the Bureau of Vector Control, California State Department of Public Health. We are indebted to their staff, especially Mr. Ernest G. Meyers and Mr. Edmond C. Loomis, and to the staff of the several mosquito abatement districts participating in the surveillance, for the collection, sorting, and identification of mosquitoes on which the work in this paper is based.

was centrifuged in the cold in an angle rotor in the Spinco machine for 15–20 minutes at 15,000 rpm, and the supernatant fluid was removed with care to avoid disturbing the sediment and the pellicle of fatty materials, which contains entrapped contaminants. The fluid, slightly more than 3 ml. in amount, was divided into two equal portions, one of which was placed into a sterile ampoule, flame-sealed and stored on dry ice, and the other used for animal and tissue culture inoculation.

Choice of cell type. In the course of other studies in this laboratory (unpublished observations) stock passage strains of WEE virus were titrated in parallel in cultures of several different cell types in order to determine the infectivity for each type of cell. A series of replicate titrations was done using both monolayer cell cultures and colorimetric methods, and employing strain HeLa, strain KB, human amnion, monkey kidney, and chick embryo cells. The titer of the WEE virus was invariably higher in chick embryo tissue than in any of the other cell types. Thus, in parallel titrations of stock viruses, there was a difference of from 4.0 to 4.5 logs in the infectivity endpoints between strain HeLa cells and chick embryo tissue. The differences in infectivity titers between monkey kidney, human amnion, and strain KB cells and the chick embryo cells were somewhat less, but still appreciable. Because the highest viral titers were achieved in chick embryo tissue, this was considered to be the tissue of choice and was used throughout these studies, although other tissues may prove to be as useful.

Preparation of chick embryo cells. Chick embryos of nine to eleven days of age were harvested in the usual manner and immediately placed into sterile phosphate-buffered saline (pH 7.4) or Hanks' balanced salt solution (pH adjusted to 7.4 with a few drops of 8.8% sodium bicarbonate solution). The embryos were thoroughly rinsed three times in large volumes of either the phosphate-buffered saline or Hanks' solution, and then grossly minced with scissors. The tissue mince was further washed in two or three changes of either the phosphate-buffered saline or Hanks' solution. After the final wash, the tissue mince was placed into a 50 ml. syringe and forced by means of the plunger through the tip of the syringe into a flask, the size of the flask depending upon the number of embryos being processed. Two additional washings served to eliminate most of the red cells and undesirable debris. After the last wash fluid was decanted, 0.25% trypsin (trypsin 1:300, Nutritional Biochemicals Co.) in Hanks' balanced salt solution was added to a depth of 1 to 1.5 inches, the actual amount added depending upon the number of embryos and the size of the flask. The tissue mince was agitated on a magnetic stirrer at low speed for a period of one hour. The resultant cell suspension was allowed to stand for three minutes to permit large particulates to settle, and the fluid was carefully decanted into 100 ml. centrifuge tubes through a 72-mesh stainless steel wire cloth. After horizontal centrifugation at 600 rpm for ten minutes, the supernatant fluid was removed by aspiration, the cell pack was resuspended in growth medium (see below), transferred to 15 ml. conical graduated centrifuge tubes and again centrifuged horizontally at 600 rpm for ten minutes. The volume of the packed cells was determined and the cell suspension was appropriately diluted according to the purpose for which it was to be used. For the preparation of monolayer cell cultures in tubes, the cell pack was diluted 1:200, and for use in metabolic-inhibition tests (colorimetric tests) the cell pack was diluted 1:125.

Cell culture medium. Preliminary studies were done with several different media, and the following combination of ingredients was found to be the most satisfactory for the purposes of the work reported in this paper.

Medium 199 (in Hanks' balanced salt solution) 47.5%. Lactalbumin hydrolysate-yeast extract medium,[2] 47.5%. Horse serum (inactivated at 56°C. for 30 minutes) 5.0%. Sodium bicarbonate (8.8%) was added to the above medium in the proportion of 1.25 ml. per 100 ml. of medium. The following antibiotics were also added: penicillin, neomycin and streptomycin, 125 units of each per ml.; bacitracin, 1.25 units per ml.; and mycostatin, 100 units per ml.

Containers used for tests. Kimble Neutraglas tubes, 16 mm. x 125 mm., were used for the preparation of monolayer cell cultures. After the appropriate amount of cell suspension had been dispensed into the tubes, they were closed with

[2] Lactalbumin hydrolysate-yeast extract medium had the following composition:

Lactalbumin hydrolysate	5.0 gm.
Yeast extract	1.0 gm.
$NaHCO_3$	1.1 gm.
Glucose	4.5 gm.
Phenol red, 1%	1.6 ml.
Earle's balanced salt solution, to	1,000 ml.

non-toxic white rubber stoppers[3] until the inoculum could be added, usually a matter of minutes. During incubation, the inoculated tubes were held in metal racks at an angle approximately 5° from the horizontal. Translucent polystyrene plates[4] were used for the metabolic-inhibition (colorimetric) tests, which are described below. Incubation temperature for both the monolyer cell and colorimetric systems was 35°–37°C.

Procedure for isolation of virus. Chick embryo cell suspension prepared and diluted as described above was placed on a magnetic stirrer and continuously agitated by means of the magnetized stirring bar. Continuous agitation served to maintain the uniformity of the cell suspension during the time required to dispense the cells into tubes. A Cornwall automatic syringe, fed from the reservoir flask holding the stock cell suspension, was used to deliver the cells into tubes; each tube received 0.9 ml. of the cell suspension. After the requisite number of tubes had been prepared, the tubes were divided into groups of four and each group of four was inoculated with material from a single mosquito pool suspension. Each tube in a group received 0.1 ml. of mosquito pool suspension.

In many cases the mosquito suspensions exhibited a marked toxic effect on the cells, evidenced by partial destruction of the cell sheet within 24 to 48 hours after inoculation. This toxic effect did not seem to hamper the growth of the virus. In view of this, as well as the fact that the toxic effect of the inoculum disappeared with subpassage, it appeared reasonable, after some experience with the method, to allow first-passage cultures to incubate for five days and then to make a sub-passage without reading the first-passage cultures microscopically for cytopathogenesis. The following procedure was consequently adopted as a routine in the latter phases of the study. Cultures inoculated with mosquito suspensions were set to incubate for five days, during which time they were not examined. On the fifth day the material from each group of four tubes was harvested and pooled; this was done by scraping any remaining adherent cells off the glass, withdrawing the fluids and suspended cells with a pipette, and pooling these materials with material similarly obtained from other tubes of the group of four. Each pool of culture material was then inoculated into another set of four freshly prepared tubes, each tube receiving 0.1 ml. The tubes representing the second passage were examined microscopically at 48, 96, and 168 hours post-inoculation. If one or more tubes of the set of four showed from 2+ to 4+ degeneration at any of these time intervals, the cells and fluids of all four were harvested, pooled and stored in a mechanical refrigerator at −20°C. until they could be examined in the identification scheme outlined below. Those tubes which showed no evidence of cellular degeneration at the two earlier examinations (48 and 96 hours) were held for a total of seven days (168 hours), at which time they were read and either passaged or discarded, depending upon the presence or absence of cytopathic changes. The same procedure was followed in the case of third passages, which were done in a number of instances and are mentioned further in the section dealing with experimental findings.

Procedure for identification of isolates. Metabolic-inhibition, or colorimetric, tests for the assay of poliomyelitis virus and antibody have been described by Salk *et al.* (1954), by Lipton and Steigman (1955) and by Robertson, Brunner and Syverton (1955). In these methods, serial dilutions of a patient's sera, for example, are tested against a known standard test dose of virus. A recent publication from this laboratory (Lennette *et al.*, 1957a) describes a colorimetric method for the identification and typing of adenoviruses; the method is based on two factors, namely, knowledge as to the expected degree of infectivity of fresh isolates for cell cultures, and the amount of specific immune serum required to protect the cells against the amount of virus expected to be present in a given dilution of the viral isolate. The following procedure is based on the same considerations. Titrations of some of the early WEE virus isolates indicated the general level of virus being encountered, and simultaneous checkerboard-type assays in a metabolic-inhibition method indicated the dilution of specific immune serum necessary to neutralize completely these isolates, as well as standard strains of the virus.

As the information presented under "Experimental" became available, it was utilized to devise a colorimetric identification test in which a standard dilution of the virus isolate was tested against an equal amount of the appropriate dilution of specific immune serum. The colori-

[3] Stopper S-124, size 0, obtained from William West and Company, Phoenixville, Pennsylvania.

[4] Available from Linbro Chemical Company, Watertown, Connecticut.

metric test, as employed at present, is performed as follows. Plastic plates (polystyrene) are used and twelve depressions, or cups, in each plate are utilized in the identification of a virus isolate. A 1×10^{-2} dilution of the virus isolate is made in the growth medium and 0.2 ml. is delivered to each of the twelve cups. To four of the cups containing the virus dilution is added an equal amount of normal rabbit serum diluted 1:32 in the growth medium. To the second set of four cups is added 0.2 ml. of WEE immune serum (rabbit) diluted 1:32 and to the third set of four cups is added 0.2 ml. of SLE immune serum (rabbit) also diluted 1:32. The serum-virus mixtures are incubated for 30 minutes at room temperature and then 0.2 ml. amounts of the chick cell suspensions are added. Finally, 0.7 ml. of mineral oil[5] is added to each cup to provide a seal. The entire plastic plate is then covered with a sheet of sterile aluminum foil, stacked with other plates, and incubated at 35°–37°C. for 5 to 7 days. The color changes taking place during this period are read and recorded on the day when cell controls have reached a pH of 6.8. A pH reading of 7.4–8.0 is taken as evidence of cell death, i.e., viral activity, and a pH reading of 6.8–7.2 as evidence of cell growth, i.e., neutralization of viral activity. Appropriate serum controls, and controls (titrations) on the number of cells employed are an essential part of the test.

EXPERIMENTAL

Inoculation of chick embryo cell cultures with mosquito pool suspensions leads to the appearance, in some instances, of a cytopathic effect. Experience gained over the course of several years during which hundreds of pools of *Culex tarsalis* were inoculated into embryonated hens' eggs and albino Swiss mice indicated that the viruses most likely to be encountered in this mosquito species in California are the Western equine and St. Louis encephalitis viruses. On this basis, it seemed reasonable to assume that cellular degeneration encountered in the cultures, if not due to an inherent toxicity of the mosquito suspensions, was a manifestation of infection by one or the other of these two viruses. In vitro neutralization tests were therefore performed to ascertain whether the cytopathogenic effect induced by the mosquito pool inoculum was due to the presence of the WEE or SLE viruses in the inoculum and if it were, to attempt to devise a simple and rapid method for the identification of these agents.

Identification of viral isolates. The first step in devising a neutralization test for rapid identification on a routine basis was, obviously, to determine the range over which the infectivity of a fresh viral isolate might be expected to occur. The second step would be to determine the concentration of specific immune serum required for complete, or almost complete, neutralization of infectivity of the isolate. From the information derived on these two points, it would be possible to determine a suitable dilution of the viral isolate to use in a neutralization test and a suitable immune serum dilution necessary to effect neutralization of this quantity of virus.

Using polystyrene plastic plates and the quantities of reagents indicated under "Material and Methods", checkerboard-type assays were done in which falling dilutions of viral isolates were tested against falling dilutions of immune serum. This served to indicate the general level of virus being encountered in the isolates and the amount of specific immune serum required to give maximal or near maximal levels of neutralization. The results of one of a number of such experiments are illustrated in Table 1, which shows that the viral isolate had a titer of $1 \times 10^{-6.5}$; that the SLE immune serum did not neutralize the agent in any of the serum dilutions used; and that complete neutralization of the agent was effected by a 1:20 dilution of the WEE immune serum, but only partial neutralization occurred in the presence of the two higher dilutions, viz., 1:40 and 1:60. In general, findings from a number of experiments showed that the infectivity level of viral isolates later shown to be WEE virus ranged from $1 \times 10^{-5.5}$ to $1 + 10^{-7.5}$ and that a 1:20 dilution of the standard WEE immune serum being used would neutralize even a 1×10^{-1} dilution, or 6.5 logs, of the virus.

To make the identification procedure as simple as possible, it appeared desirable to use only a single dilution of specific immune serum if possible, rather than several, and to use only a single dilution of the viral isolate. From the results of several experiments such as the one illustrated in Table 1, it was considered that a 1×10^{-2} dilution of the isolate would not contain excessive amounts of virus if the WEE immune serum were used in a dilution between 1:20 and 1:40. The use of sera diluted as much as possible consistent

[5] Mineral oil, white 15, obtained from Braun-Knecht-Heimann Company, San Francisco, California.

TABLE 1

Titer of virus isolates in chick embryo tissue cultures, and WEE antibody concentration required for neutralization of infectivity

Virus isolate	Immune serum		Cell growth in presence of immune serum plus virus isolate diluted							
	Type	Dil.	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
MP-108-9 2nd pass.	None		−	−	−	−	−	−	+	+
	WEE	1:20	+	+	+	+	+	+	+	+
		1:40	−	−	−	+	+	+	+	+
		1:60	−	−	−	+	+	+	+	+
	SLE	1:20	−	−	−	−	−	−	+	+
		1:40	−	−	−	−	−	−	+	+
		1:60	−	−	−	−	−	+	+	+

+ = cell growth (pH 6.8–7.2).
− = cell death (pH 7.4–8.0).

TABLE 2

Example of identification, by colorimetric method, of viruses isolated from mosquito pools in chick embryo tissue cultures

Virus isolate		Cell growth in presence of isolate diluted 1×10^{-2} and											
Number	Passage	Normal rabbit serum 1:32				WEE rabbit serum 1:32				SLE rabbit serum 1:32			
MP-124-11	1	−	−	−	−	+	+	+	+	−	−	−	−
MP-124-11	2	−	−	−	−	+	+	+	+	−	−	−	−
MP-145-22	1	−	−	−	−	+	+	+	+	−	−	−	−
MP-145-22	2	−	−	−	−	+	+	+	+	−	−	−	−
MP-158-10	4	−	−	−	−	−	−	−	−	−	−	−	−

+ = cell growth (pH 6.8–7.2) = neutralization.
− = cell death (pH 7.4–8.0) = viral activity.

with full neutralizing capacity was based on the desirability of conserving specific immune serum and the need to use antisera diluted beyond the range of possible cross-reactivity. This latter consideration is obviously not cogent insofar as the WEE virus is concerned, but might be of importance in situations where a number of related viruses are involved. Since a 1:20 dilution of the WEE antiserum neutralized a 1×10^{-1} dilution of the WEE isolates and a 1:40 dilution brought about a partial neutralization, a 1:32 dilution of immune serum was tested against serial two-fold dilutions of known WEE isolates and found to be as effective as the 1:20 dilution. Consequently, identification tests were performed as outlined under "Material and Methods", i.e., 1×10^{-2} dilutions of viral isolates were tested against 1:32 dilutions of normal and immune rabbit sera. (The use of a 1:32 dilution of SLE rabbit immune serum was reached by a similar procedure, using certain laboratory passage strains of virus.)

Table 2 presents examples of identification of viral isolates by the colorimetric neutralization tests. Each + and each − represents a cup on the polystyrene plate so that, as is indicated, four cups were used on each "point", i.e., the virus was tested in quadruplicate against each type of serum. The + indicates the occurrence of cell growth, i.e., a final pH reading between 6.8 and 7.2 colorimetrically which, in turn, points to neutralization of the virus by the specific immune serum. The minus sign conversely indicates the occurrence of cell destruction (viral activity) and is evidenced by little or no change from the original pH, i.e., a final colorimetric pH reading of 7.4 to 8.0. As will be noted from Table 2, the first four mosquito viruses representing, actually, two viruses in the first and second passages, were completely neutralized by the WEE rabbit immune serum; the SLE antiserum and the normal rabbit serum exerted no demonstrable neutralizing or inactivating effect.

The results with isolate MP-158-10 shown in Table 2 are also of interest. It will be noted that neither the WEE nor the SLE antisera had any protective effect on cultures inoculated with this agent. That cellular destruction was not due to a toxic factor is indicated by the fact that the material was in its fourth passage, by which time the toxic elements should, at least in our experience, be diluted well beyond the range of deleterious activity. The cytopathic effect in this case was due to an agent subsequently identified as a virus not infrequently recoverable from *C. tarsalis* in California and briefly described elsewhere as a new arthropod-borne virus (Lennette, 1955; Lennette *et al.*, 1957b). A number of strains of this agent have been isolated (as indicated in a later table) by the methods described above and that phase of the present studies dealing with this new virus will be reported in a separate communication.

Depending upon the passage level of the "isolate", one or another of the following possibilities must be considered when no evidence of a neutralizing effect is encountered with the immune serum employed: (a) cell destruction is due to toxic factors; (b) the infectivity of the preparation is beyond the effective neutralizing capacity of the specific immune serum used; or (c) cell death is due to the presence of a viral agent which is immunologically different from that used to prepare the immune serum.

Application of tissue culture methods to isolation of WEE virus from mosquito pool suspensions. In any attempt to develop a tissue culture method for the isolation and identification of viruses, several practical considerations have an important bearing if the technique is to find more than a restricted research application. Thus, the method should, for example, be as highly specific as possible with respect to viral activity, i.e., insofar as possible, cytopathogenesis should reflect the presence of virus and not the presence of nonspecific toxic elements. (Although in the present work, considerable difficulty was encountered with toxicity, as will be shown further below, this difficulty may have been referable to the diluent employed for the processing of mosquitoes and possibly can be largely obviated by the use of another type of diluent—see Discussion.) Also, the cell culture, or test, system should be highly sensitive to the presence of virus especially when it is present in minimal amounts. In the experimental procedures reported here an attempt was made, as will be shown in later tables, to define within the limits of this system the manipulations required to detect the presence of virus in as high a proportion of the mosquito pool specimens as possible. It is desirable, furthermore, to keep the techniques as simple as possible; in the methods described in this paper the inoculum was added to the cells without waiting over the several days required to produce a monolayer culture of cells, and a colorimetric method was used for the identification of the re-recovered viruses. Finally, and of some consequence, there is the matter of the rapidity with which the method can give an answer. One objective in developing the method described here was to obtain identification of viral isolates at as low a passage level as possible.

The ideal system of course would be one in which virus, even if present in the inoculum in trace amount, could be recovered in the initial passage and identification of the agent made from the infected fluids of this first culture passage. In practice, however, such a situation is seldom encountered at the present time and the technique described in this paper is no exception. As indicated earlier, considerable difficulty was experienced in the initial passages of mosquito pool suspensions with cellular degeneration induced by factors other than viral growth. This non-specific cytopathogenesis, also seen in other tissue culture systems inoculated with field materials, is attributable to cyto-toxic substances present in the test material itself or in the suspending menstrua used. With sub-culture, the toxic factor(s) is diluted and seldom constitutes a source of difficulty in later passages. In contradistinction to the occurrence of specific or non-specific cellular degeneration, there exists the possibility that materials containing minimal amounts of virus may not bring about an apparent cytopathogenic effect within the life span of the cell culture. To circumvent such a possible situation, sub-cultures become a requisite part of the test procedure.

In order to determine the extent to which sub-culturing was required to eliminate cytopathogenic effects due to toxic factors, and to reveal the presence in the inoculum of virus not detectable on initial passage, 294 mosquito pools were studied and the findings are summarized in Table 3. It will be noted that of the 294 mosquito pools tested, 92, or 32 per cent, exerted a definite toxic effect on the chick embryo cells of the first

passage culture. The effect of sub-culture on toxicity of the inoculum is illustrated in Table 3 by the fact that only 12, or less than 5 per cent, of the 294 pools exerted a demonstrable toxic effect in the second passage. Although not shown in the table, third passage cultures were completely free of the influence of toxic materials present in the original inoculum. It will also be noted that in 23 instances specific viral cytopathogenesis was noted in the first passage and was recorded as such. This is over and above the 92 instances in which cytopathogenesis regarded as non-specific in nature was encountered. Colorimetric neutralization tests were performed with the fluids from 16 of the 23 mosquito pool cultures considered to have shown a specific cytopathogenic effect and the cytopathogenic agents were identified as WEE virus. Further proof of the identity of these cytopathogenic agents as WEE virus was obtained by subsequent testing of second-passage fluids. The remaining seven cultures were tested at the second-passage level and found to contain WEE virus.

TABLE 3

Proportion of strains of WEE virus isolated from mosquito pools (Culex tarsalis) *in 1st passage and proportion of additional recoveries achieved by one additional passage. (Chick embryo tissue cultures.)*

Mosquito pool-collection no.	No. pools tested	No. toxic in 1st pass.	No. isolations in 1st passage	Additional isolations with 2nd passage	No. toxic in 2nd pass.	Total no. isolations
104	16	5	0	0	0	0
105	17	13	0	0	1	0
107	38	20	0	0	0	0
108	40	0	0	1	0	1
109	44	0	2	1	7	3
112	43	32	1	0	1	1
120	17	14	3	2	1	5
124	35	2	8	3	2	11
145	44	6	9	2	0	11
Totals...	294	92	23	9	12	32

The differences between non-specific degeneration and specific degeneration due to viral activity are not easy to define; while ability to make a distinction between these two types of cellular destruction is gained with experience, such distinctions are not always possible. The results in Table 3 with reference to the findings obtained on the first passage of the mosquito pools show that the primary passage does not, in a high proportion of instances, produce cultural changes which can be unequivocally interpreted as to specificity and thus be applied practically; a second passage was required in order to reduce the toxic effects to a level where they did not interfere too greatly with interpretation of the findings. In addition, a second passage served to increase the sensitivity of the method by detecting the presence of virus in mosquito pool specimens which were negative on the first passage. Thus, the second passage yielded nine additional recoveries of WEE virus to give a total of 32 isolations from 294 mosquito pools. The data in Table 3 may be summarized as indicating that under the conditions employed, at least two passages of the arthropod material are required—the second passage increases the sensitivity of the method for the detection of virus as well as increasing its specificity.

To determine whether any further advantages in the detection of viral agents would accrue with sub-culturing beyond the second passage level, a series of 222 mosquito pool suspensions was subjected to three passages in chick embryo culture systems. The results of this experiment are shown in Table 4. It will be noted that a total of 35 strains of WEE virus was isolated by the end of the second passage of these 222 pools. A third passage of the negative materials did not result in the recovery of a single additional strain of virus. For example, in the case of mosquito pool collection No. 145, a total of 44 pools was tested and 11 strains of WEE virus were isolated in either the first or second passages; sub-passage of the remaining 33 negative pools did not result in isolation of any additional strains. While it is not impossible that the use of third-passage subcultures might result in the isolation of an occasional additional strain of virus, the data in Table 4 indicate that the time and labor involved constitute a disadvantage which outweighs any advantages associated with this procedure. The findings in Table 4 also strengthen the impression previously gained and discussed just above, that two passages of the mosquito material comprised the optimal approach from both the standpoint of sensitivity and specificity.

It should be emphasized that the statement that two passages of mosquito pool material are optimal is applicable at this stage of our knowledge only to the WEE virus. As mentioned at the

TABLE 4

Relative effectiveness of second and of third passages of mosquito pool materials (Culex tarsalis) *in the isolation of WEE virus in chick embryo tissue cultures*

Mosquito pool-collection no.	Number pools tested	Number strains isolated by 2nd passage	Additional isolations with 3rd passage
145	44	11	0
158	47	8	0
159	37	8	0
160	47	5	0
621	47	3	0
Totals.....	222	35	0

TABLE 5

Isolation of viruses from Culex tarsalis *by means of chick embryo tissue cultures. California, 1956*

Mosquito pool-collection no.	Number of pools tested	No. of virus isolations		Presence WEE virus first established in passage level		
		WEE	Other*	1	2	3
104	16	0	0			
105	17	0	0			
107	38	0	0			
108	40	1	0	0	1	ND†
109	44	3	0	2	1	ND
112	43	1	1	1	0	ND
120	47	7	0	3	4	ND
123	34	8	0	—‡	8	ND
124	35	11	0	8	3	ND
145	47	11	2	9	2	0
158	48	8	1	—	8	0
159	40	8	3	—	8	0
160	48	5	1	—	5	0
621	48	3	1	—	3	0
Totals...	545	66	9	23	43	0

* An apparently new arthropod-borne virus, described elsewhere (6, 7).

† ND = Not done.

‡ — = Tubes not examined for cytopathogenic changes.

beginning of this paper, we have had as yet no experience with naturally-occurring SLE virus and so do not know what factors would play a role in the isolation of this virus in tissue culture systems. That modification of the procedure described here will be required depending upon the agent under study was indicated during the course of this work. On a number of occasions an agent, subsequently found to be neither the WEE nor SLE viruses, was isolated. The use of three passages of the mosquito pool suspensions appeared to be an important requisite for the recovery of this agent, since a number of isolations were made at the third passage level whereas the initial and the second passages had given no evidence that a viral agent was being propagated. This agent is the one which has been referred to above as a hitherto undescribed arthropod-borne virus (Lennette *et al.*, 1957b).

In Table 5 are summarized the results obtained in testing pools of *C. tarsalis* collected during the 1956 mosquito surveillance season. Of the 850 pools of *C. tarsalis* collected during the season, 545, representing fourteen different weekly collections, were tested in chick embryo cell cultures. As is shown in Table 5, 75 of the 545 pools yielded a virus. Colorimetric neutralization tests showed that 66 of the isolates were WEE virus, and 9 represented the new arthropod-borne virus mentioned above. No isolations of SLE virus were made. Of the 66 strains of WEE virus recovered, 23 isolations were made at the first-passage level and sub-culturing of negative or toxic material yielded 43 additional strains at the second-passage level. Third passage of the negative materials did not lead to recovery of any additional strains of WEE virus.

DISCUSSION

The tissue culture method described in this paper represents a simple and practical method for the recovery and identification of naturally-occurring viruses from their mosquito host. We believe that the use of this method should serve to expedite greatly the examination of mosquitoes and perhaps other arthropods in connection with epidemiologic studies of arthropod-borne viral diseases.

Inoculation of mosquito suspensions into chick embryo cell cultures was followed in many instances by cellular destruction which proved to be due to toxic factors in the inoculum and which could be eliminated by sub-culture through one or two additional passages. This nonspecific degeneration was traced to the diluent used for suspending the mosquito pools. It has been found that if instead of normal rabbit serum a diluent consisting of a 0.75 per cent crystalline bovine albumin (fraction 5) in phosphate-buffered saline is used, relatively little cyto-toxicity occurs even in the initial passage.

While a second passage was necessary in about ⅓ of the tests to reveal the specific cell changes produced by viral activity, it was found in the case of WEE virus, that little was to be gained by a third subculture since no additional strains of the virus were recovered in this way. No strains of SLE virus were isolated, and what its behavior would be in this tissue culture system is unknown. Three passages, however, appeared to be important for the isolation of a third agent, hitherto undescribed, which was neither WEE nor SLE virus, and whose presence was unsuspected in the initial and second passages (Lennette *et al.*, 1957b). The colorimetric system devised to identify viral isolates required only a single dilution of the isolate, and a single dilution of specific immune serum.

During the 1956 season, 66 strains of WEE virus and 9 strains of a new arthropod-borne virus, a total of 75 viruses, were recovered from 545 pools of *C. tarsalis* by the tissue culture system. At the same time 139 strains of WEE virus were recovered from 850 pools of the same mosquito by the use of adult mice and embryonated hens' eggs. This does not constitute a valid comparison of the two methods since there was a considerable disparity in the number of mosquitoes examined, and comparative studies, to be meaningful, should be done simultaneously by the same observers. In several instances, WEE virus recovered through the use of mice or embryonated eggs was not recovered, or was missed, in the tissue culture system. While it is possible that the discrepancy reflects differences in sensitivity of the test systems used, there is also the possibility that the much greater familiarity associated with the large-scale use of the mouse and embryonated egg in field studies of the arthropod-borne viruses contributed significantly to recognition of viral activity in these hosts. We hope, however, to conduct a comparative evaluation of the usefulness of the mouse, the embryonated egg and the chick embryo tissue culture system for the isolation of viruses from arthropods, primarily *C. tarsalis*, collected in the field.

With respect to simplicity and ease of handling, the tissue culture method utilized here appears to have a number of advantages over the use of the usual test hosts, i.e., the mouse and the embryonated egg. The large number of test cultures which can be prepared from the tissues of several chick embryos, the ease with which they are inoculated, stored and read, constitute highly practical considerations; the advantages are obvious when one considers the space requirements, feeding and maintenance needs and subpassage operations associated with animal and egg work.

SUMMARY

A chick embryo tissue culture method for the isolation of viruses from mosquitoes, and perhaps other arthropods, is described. The application of this method to mosquito surveillance studies concerned with the prevalence of WEE and SLE virus infections in *C. tarsalis* led to the recovery of 75 virus strains from 545 pools of this arthropod. Of the 75 agents, 66 proved to be WEE virus and 9 were found to represent a new arthropod-borne virus previously described from this laboratory. No recoveries of SLE virus were made.

Because of the high incidence of non-specific cellular degeneration encountered in the inoculated cultures, sub-passages were necessary. Presumably, only two passages of the mosquito pool suspensions were required to recover all of the strains of WEE virus detectable by this method, since additional sub-culture through the third passage did not yield any additional strains. This did not hold true in the case of the new arthropod-borne virus, as it was found that the presence of this agent became apparent in a number of instances only in third passage materials. It thus seems conceivable that decisions as to the number of passages to be undertaken routinely in any given epidemiologic or field study may depend upon the pathogenetic properties of the virus under study.

A method was devised for the rapid colorimetric identification of the WEE virus. The simplicity of the method resides in the fact that a single dilution of the viral isolate is tested against a single dilution of specific immune sera.

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THE COMPARATIVE VALUE OF EMETINE AND CHLOROQUINE IN AMEBIC LIVER ABSCESS

A. J. WILMOT, S. J. POWELL AND E. B. ADAMS

Department of Medicine, University of Natal and the Amoebiasis Research Unit[1]

Since Conan (1948) showed that chloroquine was effective in the treatment of hepatic amebiasis, the activity of the drug has been confirmed by numerous reports, and many now believe that chloroquine is the drug of choice in this condition, chiefly because it is held to be as efficacious as emetine hydrochloride but less toxic (Hoekenga and Maximo, 1950; Patterson and Lawlis, 1956). Few comparative studies have been made. Harinasuta (1951), however, has compared emetine and chloroquine in two groups of 45 patients suffering from amebic liver abscess and concluded that "chloroquine appeared to possess nearly as much curative value as emetine in the treatment of amebic liver abscess. It might be somewhat inferior on the point of relapse rate."

The purpose of the present study is to assess the relative efficacy of emetine and chloroquine in the treatment of amebic liver abscess.

MATERIAL AND METHODS

Alternate male African patients diagnosed as suffering from amebic liver abscess were given emetine or chloroquine. The diagnosis was established in all cases by aspiration within three days of admission, and in all the pus removed was characteristic and bacteriologically sterile. The finding of an associated disease excluded a patient from the series. All patients were kept under observation in hospital for 30 days and thereafter they were asked to report for follow-up at 1 month, 3 months and 6 months after discharge.

Emetine hydrochloride was given by intramuscular injection in a daily dose of 1 grain. Each patient received two courses, the first lasting 10 days and the second 6 days, with an interval of 14 days between courses. Chloroquine was given as the diphosphate (250 mgm. tablets equivalent to 150 mgm. of the base) or the sulphate (200 mgm. tablets equivalent to 150 mgm. of the base) in a dose of 4 tablets immediately, followed by 2 tablets 6 hours later and then 1 tablet twice daily for 29 days. In addition, all patients received 600 mgm. diiodohydroxyquinoline thrice daily for 20 days.

When there was active amebic dysentery in addition to liver abscess in cases receiving chloroquine, we added tetracycline in a dosage of 1 gram daily for 10 days. We did this because we felt that treatment with chloroquine and diiodohydroxyquinoline is inadequate for active bowel disease, and because we believe that tetracycline is ineffective in amebic liver abscess (Wilmot *et al.*, 1952; Wilmot, 1956). At the outset it was decided that both emetine and chloroquine would be given if a patient did not respond or deteriorated on treatment with one of these drugs alone, and that if a patient relapsed after leaving hospital he would be given the drug he had not previously received.

The series consisted of 19 patients treated with emetine, 5 of whom had amebic dysentery as well as liver abscess, and 16 treated with chloroquine 3 of whom had associated dysentery.

RESULTS

One patient treated with chloroquine was given emetine after 10 days as it was felt that he was deteriorating clinically and that it was unjustifiable to continue with the single drug. A further case in the chloroquine series had not responded fully after 32 days and was then given emetine. Both recovered satisfactorily.

Apart from these 2 cases there was no apparent difference in the initial response to these two drugs. The relapse rate however was significantly different. In the emetine series there were no relapses, but among the patients treated with chloroquine, 4 returned within 6 weeks with symptoms and signs of liver abscess and pus was obtained at aspiration. These 4 patients were treated with emetine with a satisfactory result.

As is usual with African patients the follow up was incomplete. In the chloroquine series, apart from the relapses, 4 patients reported at 1 month

[1] The Amoebiasis Research Unit is sponsored by the following bodies: The South African Council for Scientific and Industrial Research, The Natal Provincial Administration, The University of Natal, The United States Public Health Service (Grant E/1592).

and one of these again at 3 months. They were assessed as "cures". In the emetine series 11 patients returned at 1 month, 4 again at 3 months and 2 again at 6 months. All were well.

Apart from a moderate rise in pulse rate in some of the patients treated with emetine no toxic reactions were encountered with either drug.

DISCUSSION

Amebic liver abscess is a dangerous condition which may terminate fatally, usually by rupture of the abscess into adjoining viscera or from secondary bacterial infection. For this reason treatment should not only be effective in immediate control but reduce the incidence of relapse to a minimum.

In the doses used in this trial, chloroquine appears to be as effective as emetine in the alleviation of the initial symptoms, but it carries the risk of a significant relapse rate which we did not find with emetine. Although the follow up obtained was unsatisfactory, particularly in the chloroquine series, this does not invalidate the results as the African is reluctant to return to hospital when he is well. However, if symptoms recur, he is likely to return to this hospital as there is no alternative free medical service in the area.

The growing preference for chloroquine in the treatment of hepatic amebiasis is largely based on the fear of toxic reactions to emetine, but provided this drug is not used in patients with heart lesions and the dose is reduced in the young and the aged, we have not experienced serious toxic reactions in these or other patients who number many hundreds. Two courses of emetine were used because it has been shown that a single 10-day course carries a relapse rate of 7.7 per cent which a second course reduces to less than 1 per cent (Wilmot, 1949). The dose of chloroquine was that usually recommended in the literature, and it is felt that the period of administration was adequate.

We think that there are grounds for caution in recommending the substitution of chloroquine for emetine in the treatment of amebic liver abscess, and that the disease represents a greater risk to the patient than the possible occurrence of emetine toxicity. Chloroquine might prove more effective in a higher dose, but Wilkinson (1953) has reported a number of side effects when 450 mgm. of the base were given daily. It might also be possible to achieve satisfactory results by combining emetine and chloroquine and so reduce the total emetine dosage.

SUMMARY

Patients in whom the presence of an amebic liver abscess was confirmed by the aspiration of characteristic pus were treated in two groups with emetine or chloroquine. Of 19 cases given emetine all responded well initially and none relapsed. Sixteen patients were treated with chloroquine. Initial response was poor in 2 and they were given emetine with a satisfactory result. Four of the remaining 14 patients returned within six weeks with a recurrence of the abscess.

ACKNOWLEDGMENT

We wish to thank Dr. S. Disler, Medical Superintendent of King Edward VIII Hospital, for facilities.

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A SIMPLE QUALITATIVE TEST FOR CHLOROQUINE IN URINE

WILLARD T. HASKINS

National Institutes of Health, National Institute of Allergy and Infectious Diseases,[1] *Bethesda, Maryland*

This test was developed to offer a rapid method of detecting chloroquine in urine samples under field conditions, where it may be used as a means of determining whether a population is ingesting the drug. The test is based on the method described by Brodie and Udenfriend (1945) for the estimation of basic organic compounds in biological material by salt formation with methyl orange.

REAGENTS

Sodium hydroxide solution, 10 per cent.

Ethylene dichloride, purified grade. Chloroform may be substituted for this reagent if necessary.

Methyl orange solution. Prepare a boric acid solution by dissolving 5 gm. of boric acid in 100 ml. of water. To this solution add 0.1 gm. of indicator-grade methyl orange and shake the mixture for a few minutes. The solid dissolves rapidly, followed in a few minutes by the formation of a precipitate. Allow the solution to stand for a few hours or overnight and then filter it. The reagent is stable for some time. If a precipitate forms, it may be removed by filtration without loss of efficiency.

SAMPLES

In addition to the urine samples to be examined, it is necessary to have a control urine sample from a person who has not been taking the drug or any other basic substance which would interfere with the test.

PROCEDURE

Place 5 ml. of urine in a test tube. Add approximately 1 ml. of sodium hydroxide solution and 5 ml. of ethylene dichloride. Stopper the tube and shake it for about 1 minute. Allow the layers to separate. This may take some time with samples which emulsify. If a centrifuge is available, it can be used to separate the layers rapidly. With a capillary pipette fitted with a rubber bulb, draw off the ethylene dichloride layer and transfer it to a clean test tube. Avoid transferring any of the aqueous layer with the solvent. Only 2.5 to 3 ml. of the solvent need be recovered at this point. Add approximately 0.5 ml. of the methyl orange solution to the ethylene dichloride layer, stopper, and shake the tube for 15 to 20 seconds. Allow the layers to separate. A definite yellow color in the ethylene dichloride layer as compared to the control tube from urine containing no drug indicates the presence of chloroquine.

SENSITIVITY

Urine containing 2 mg. per liter of the drug gives a perceptible test; 5 mg. per liter produces a very definite yellow color; and 10 mg. per liter an intense yellow. No substances found in normal diets would be expected to interfere with the test. However other basic drugs such as quinine and other cinchona alkaloids, plasmochin, codeine, benzedrine, ephedrine, and demerol, might interfere if present in the urine in sufficient quantity.

TRIAL EXPERIMENT

Eight white males each took 300 mg. of chloroquine base (Aralen) in a single dose. Urine samples were collected hourly for 5 hours and then twice daily for 13 days. The drug appeared in the urine rather abruptly 4 to 5 hours after the dose. The test was strongly positive for the first 4 to 5 days and then declined in intensity to the 10th day when it was barely distinguishable from the blank. By the 12th day all specimens were negative. Thus, at the recommended dosage of 300 mg. of base once a week, the urine should give a positive test continuously throughout the regimen.

APPLICATION TO OTHER ANTIMALARIAL DRUGS

The test was found to be unsatisfactory for the detection of amodiaquine (Camoquin) after a single dose of 300 mg. The amodiaquine-methyl orange complex is nearly colorless and cannot be distinguished from the blank. Pyrimethamine (Daraprim) was also undetectible in the urine

[1] Laboratory of Tropical Diseases.

after a single dose of 25 mg. Here the difficulty lies in the extremely small amounts present, since it was shown that pyrimethamine added to the urine in a concentration of 2 mg. per liter gave a positive test.

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ACUTE AFRICAN AMEBIASIS: CLINICAL-LABORATORY STUDIES AND RESPONSE TO MA-307[1]

HAMILTON H. ANDERSON, ALEXANDER J. WILMOT, LEONARD FREEDMAN AND JEANETTE VAN D. ANDERSON

University of California, San Francisco, California; The Natal Provincial Administration and University of Natal, Durban, South Africa

The opportunity to visit and work in the Amoebiasis Research Unit, in Durban, South Africa, was afforded two of us (H. H. A. and J. Van D. A.) during April–May, 1957. The purpose of this visit was to collaborate with Dr. A. J. Wilmot in clinical studies and Dr. Leonard Freedman, under the direction of Dr. R. Elsdon-Dew, Honorary Director. Studies initiated at the University of California in San Francisco have been focused on relating laboratory examinations of patients having drug-refractory (chronic) amebiasis, under institutional conditions,[2] to therapy with an amebicide (MA-307)[3] (Miles-Ames Research Laboratory, Elkhart, Indiana) which, in the experimental laboratory, proved to be effective. A similar study was suggested by Dr. R. Elsdon-Dew in Bantus having acute intestinal amebiasis with demonstrable *Entamoeba histolytica*, and thus found to be satisfactory subjects for evaluating new drug therapy.

EXPERIMENTAL THERAPY

The criteria adopted for this study have been used by Armstrong, Wilmot, and Elsdon-Dew (1949) over the past ten or more years. Since amebic ulceration of the colon is an extremely common and intractable disease in the African, and frequently devastating, it was necessary to select patients carefully to provide some uniformity in test procedure. All patients were in hospital throughout the period of study.

Bantu patients were male, ranging in age from 19 to 51 years. Their dysentery, with blood and mucus, began from 3 to 28 days before hospital entry. Approximately one-half had palpable, sometimes tender livers and one-third had a past history of amebiasis. Three-quarters of the patients had abdominal signs, usually referable to the lower bowel.

Stool examination and sigmoidoscopy were done initially, and stools were re-examined daily during 27 days in hospital, utilizing wet-mount and iron-hematoxylin staining techniques. Sigmoidoscopy was repeated at 5-day intervals. Despite variation in cases, previously defined criteria for selection of patients were used (Elsdon-Dew, 1954–1955). These were: (1) active hematophagous amebae must be demonstrated; (2) those passing only cysts were not included; (3) typical amebic ulceration must be visible on sigmoidoscopy; (4) no apparent complications, such as impending abscess of the liver, should be present.

In addition, scrapings of visible ulcers were made at the time of sigmoidoscopy, and these, together with tissue samples from ulcerated areas, were examined microscopically for *E. histolytica*. These examinations were correlated with complement fixation (CF) tests and other attempts to obtain evidence of systemic involvement. While biopsied specimens revealed *E. histolytica* in most cases ($^{2}/_{5}$), in only three of these patients was the serum satisfactory for CF test; these were positive.[4]

Assessment of results (Wilmot, 1956) was based on sigmoidoscopy and microsocpic examination, either of stools or scrapings taken from the ulcers, and the following categories were established: (1) *Success:*—symptom-free, ulcers healed, no amebae found; (2) *Probable failure:*—ulcers not healed but no amebae found either in stools or in scrapings from ulcers; (3) *Absolute failure:*—open ulceration and trophozoites of *E. histolytica* found; (4) *Cyst-passers:*—ulcers healed but cysts found in stools after completion of treatment; (5) *Removal from trial:*—cases showing

[1] Supported, in part, by the National Institutes of Health, Bethesda, Md., and the Miles-Ames Research Laboratory, Elkhart, Indiana.

[2] At Sonoma State Hospital, Eldridge, California, Dr. Thos. L. Nelson, Medical Director, has actively collaborated with one of us (H. H. A.).

[3] N,N[1]-dipiperonyl-5,11-diamino pentadecane-diHCl.

[4] We are indebted to Sister M. Ann Josephine, Salt Lake City, Utah, for these data.

TABLE 1

Drug-parasite response to MA-307

Case no.	Sigmoidoscopic and parasitic findings after R_x (days)					Additional treatment†
	5	10	15	20	27	
125	Open ulcers, trophozoites*	Open ulcers, trophozoites	Open ulcers, cysts*	Ulcers healed, cysts	Healed, stool negative	Given Emetine 0.065 Gm. Ⓑ 10X after 10 days
127	Ditto	Ditto	Open ulcers, stool negative	Open ulcers, trophozoites	Ditto	Ditto
130	Ditto	Open ulcers, stool negative	Ditto	Ditto	Ditto	Ditto
133	Ditto	Open ulcers, trophozoites	Open ulcers, trophozoites	Open ulcers, stool negative	Ditto	Ditto plus Phthalylsulfathiazole and Diodoquinoline (at 15 days).
137	Ditto	Ditto	Open ulcers, stool negative	Healed, stool negative	Ditto	Given Emetine 0.065 Gm. Ⓑ 10X after 10 days
143	Ditto	Open ulcers, stool negative	Healed, stool negative	Ditto	Ditto	
144	Open ulcers, stool negative	Open ulcers, trophozoites	Open ulcers, stool negative	Healed, cysts & trophozoites	Healed, cysts & trophozoites	Ditto
145	Open ulcers, stool negative	Open ulcers, stool negative	Open ulcers, cysts	Ditto	Ditto	
148	Open ulcers, trophozoites	—	—	—	—	Deteriorating clinically, ∴ given Tetracycline, Diodoquin & Chloroquine.
149	Ditto	Open ulcers, trophozoites	Open ulcers, cysts	Open ulcers, cysts	Healed, stool negtive	Given Emetine 0.065 Gm. Ⓑ 10X after 10 days
150	Ditto	Ditto	Ditto	Ditto	Healed, cysts	Ditto

* of *E. histolytica*.

† Since MA-307 did not heal ulcers in any patient (at end of 10 days) and cleared amebae in only 3, the indicated schedules were instituted.

clinical deterioration while on treatment, or toxic symptoms. These several criteria give, in the opinion of one of us (A. J. W.), evidence of *immediate* effect of the drug under study in this disease. They do not, however, give adequate information as to permanence of the results, since African people seldom return to hospital while free from symptoms.

Table 1 summarizes the drug-parasite and patient response. A simple comparison with emetine alone might be shown at the end of 27 days of hospitalization, as indicated in Table 2.

It should be emphasized that emetine was instituted, after MA-307, in seven cases in which open ulcers persisted, and in which *E. histolytica* trophozoites were found. Thus, it may be argued that emetine alone, at 50 per cent higher dosage, might have produced a similar result. It is not

TABLE 2

Comparison of response to MA-307 and emetine

Treatment and duration	Clearance of amebae and degree of clinical response at 27 days*				
	Ulcers healed, no amebae	Not healed, but negative	Open ulcers, but positive	Ulcers healed, cysts†	Clinical deterioration
Emetine: 0.065 Ⓗ daily for 15 days (Wilmot, et al., 1949)*	18/50	7/50	11/50	14/50	—
MA-307: 0.6 gm orally each day for 10 days (In 7 cases Emetine given 10 days, after MA-307)	6/11	0/11	0/11	3/11	2/11†

* As outlined by Wilmot, et al., 1956.

† Requiring other therapy.

certain that the results obtained with the combination of MA-307 and emetine were any better than we might have obtained in such a small series using emetine alone.

No toxic signs or symptoms of a serious nature were noted after MA-307 however, except vomiting initially in one patient who, on reduced dosage, completed therapy. In a second case, bloody diarrhea occurred which may have been due to the disease since it cleared on completion of the therapy. Eight of these patients (Table 1), as well as three of five others who had from five to ten days of MA-307 therapy under less well controlled conditions, had healed ulcers on leaving the hospital, and all but two were freed of the trophozoites of *E. histolytica*. One patient became an asymptomatic cyst-passer. No information has been obtained on the number of recurrences after discharge from hospital.

CLINICAL-LABORATORY FINDINGS

As a corollary to the purpose of evaluating MA-307 in acute amebiasis, it was possible also to include a more extensive panel of function and other laboratory tests then hitherto performed in a group of patients having amebiasis. Since certain detection of systemic infection (beyond the tissues of the lower bowel) still eludes most investigators, every effort was made to study as many potentially useful reactions as feasible. The clinical laboratory findings, including serum electrophoretic pattern, were related to direct observation of the sigmoid area as previously described, in patients having *E. histolytica* infections.

Earlier studies had shown the cephalin flocculation test to be of some value as an adjunct in appraising systemic infection (H. H. A.) at Sonoma, California, and in Colombia, South America. In South Africa, however, it was of little value, as compared with other tests. In former studies, approximately 50 per cent were positive; in the latter, approximately 20 per cent. On the other hand, the CF test (not relied upon as helpful in California) has been reported positive in 60 per cent of Bantus having acute intestinal amebiasis (R. Elsdon-Dew, 1956). In such patients with amebic abscessed livers, almost 100 per cent had positive CF tests, according to this author. Since no other such comparisons were possible between the two geographic groups, it was deemed desirable due to Dr. S. P. Powell's[5] interest in serum-protein electrophoretic pattern in various disease states) to include such tests in this study. Therefore, examinations were made of zinc-sulfate and thymol turbidity, and albumin and globulin levels, as well as biopsy of sigmoid ulcers or hepatic tissue. Bantus were all passing trophozoites of *E. histolytica* and had acute dysenteric signs and symptoms at the beginning of study.

Inspection of Chart I will reveal that, while no single laboratory test proved consistently reliable, more than one observation on each patient was abnormal in half or more of patients on which we have fairly complete data. These results should be related to "values found for normal Bantu males, aged 20–42" (Kinnear, 1956).

Serologic results are quite unpredictable in acute amebic dysentery (Moan, 1957), since antibody formation takes time. Changes imply invasion of tissue by an infectious agent. Sera

[5] Member of the Medical Faculty, University of Natal, Durban, South Africa.

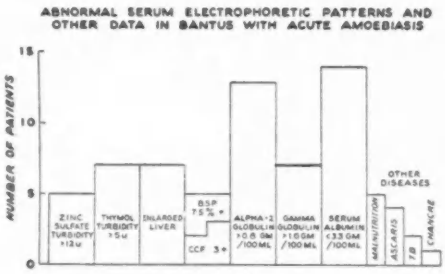

CHART I

obtained early in the disease, shortly after initial invasion, generally are negative. Moan's report of a precipitation test[6] may be of some value.

It is possible that these tests relate to some abnormality in the globulin fractions of the serum of patients having acute intestinal amebiasis. Elevation of alpha-2 fraction and gamma-globulin fraction has been noted previously (in the U. S. A.) in cases of acute infection (Stauber, 1954), notably malaria. It would be helpful if a confirmatory test would indicate, beyond reasonable doubt, that systemic invasion by the ameba has occurred. Such an objective diagnostic aid would tend to clarify a generally confused clinical impression.

Since Bantus in the present study suffered from malnutrition and a variety of other ills, the amebae cannot be held responsible for all abnormal reactions noted. In five of fifteen patients, the albumin-globulin ratio was 1:1 or higher. Other diseases, such as ascariasis and a "chancre" occurred in a minority of cases. However, several patients in this series had no debilitating associated disease.

It is suggested that more complete clinical-laboratory tests be included in initial clinical trials of new amebicides. Increased globulin levels occur in acute African amebiasis and, barring nutritional factors, may characterize this disease in addition to those parasitic disease previously reported by Stauber (1954).

SUMMARY

1. MA-307 in 600-mg daily, peroral doses over 10 days (followed by emetine hydrochloride, 0.065 gm injected daily for ⅔ usual total dosage in 7 patients) cleared 9 of 11 Bantus of *E. histolytica* and healed sigmoid ulcers in all during 27 days observation in hospital.

2. MA-307 used alone in acute dysentery had very little, if any, therapeutic effect after ten days of trial.

3. Increased alpha-2-globulin and gamma-globulin levels and some disturbance of zinc-sulfate and thymol turbidities were more common than abnormal cephalin flocculation or other tests in Bantus. Some enlargement of liver was encountered, but hepatic involvement was not obvious.

[6] Moan titrates raw antigen against a standard antigen, using a stock serum originally obtained from a case of amebic hepatitis.

ACKNOWLEDGMENTS

We are indebted to Sister M. Ann Josephine and Dr. Crichton MacNeil, of Holy Cross Hospital, Salt Lake City, for confirmatory data on serum-electrophoretic pattern, as well as complement fixation tests. Dr. Joubert's laboratory in Durban, through the kindness of Mr. Hunter, provided original data and advice on serum-electrophoretic patterns in Bantus. Dr. Daniel Widelock, Department of Health, New York City, kindly performed the complement fixation tests reported.

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THE EPIDEMIOLOGICAL FIELD KIT

DONALD L. PRICE

Walter Reed Army Medical Center, Washington, D. C.

A new epidemiological field kit was reported at the 1956 Annual Meeting of the American Society of Tropical Medicine and Hygiene (Price, 1956). In the opinion of the author requests for information on the kit have been numerous enough to warrant publication of the design and its use so that it may be duplicated by other workers.

The basic design is similar to that of a suitcase with outside dimensions of 20 by $18\frac{3}{4}$ by 8 inches and a carrying handle at the top (Figs. 1 and 2). The kit is constructed of sheet aluminum riveted at the seams. A piano hinge for opening is placed about $\frac{5}{6}$ of the way down one side instead of at the bottom, allowing a full 8 by $18\frac{3}{4}$ inch base (Figs. 1 and 2). The four corners of the base are reinforced with corner trunk brackets. The open side when supported by two small folding legs and covered by a sheet of aluminum becomes a 16 by 18 inch flat working surface. The working surface lifts up from the outside edge for access to four equal storage compartments, each $3\frac{3}{8}$ by 8 by $9\frac{3}{8}$ inches (Figs. 1 and 3). The section at the base of the hinged side is a long compartment $3\frac{1}{2}$ by $3\frac{1}{2}$ by $18\frac{1}{2}$ inches in which pipettes and other such long pieces of equipment may be carried.

Two shelves extend across the stationary side of the case providing a base for a drawer or a centrifuge-tube rack (Figs. 1 and 3). The front edges of the shelves are folded under for strength. The lower portion of this side is divided into five compartments $3\frac{3}{4}$ inches wide for carrying bottles or containers for liquids. The drawer and rack are designed to fit on the shelves and are interchange-

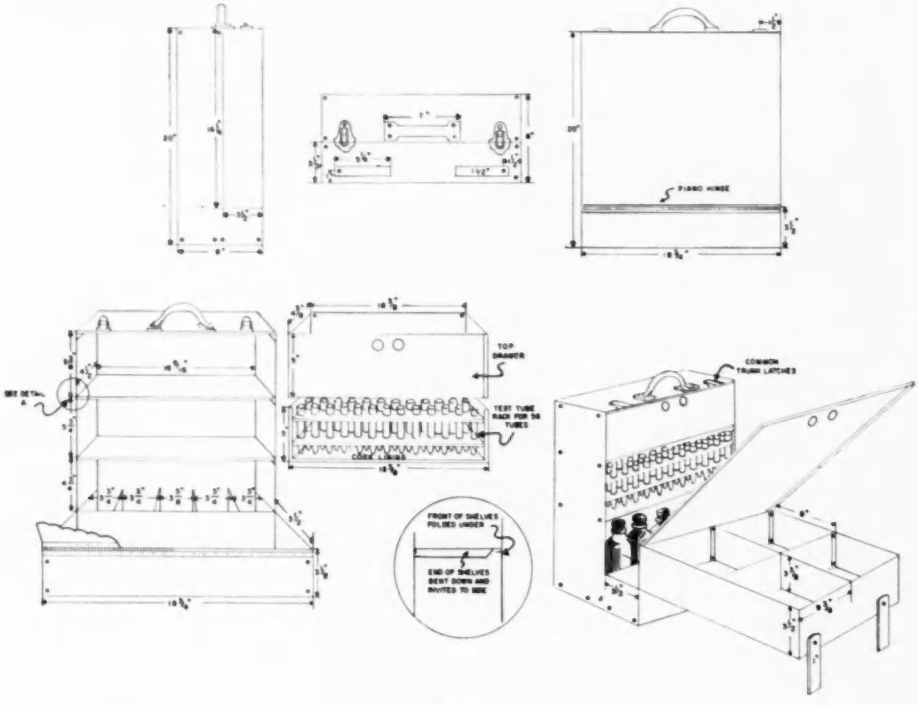
PIANO HINGE
TOP DRAWER
TEST TUBE RACK FOR 24 TUBES
CORK LINER
FRONT OF SHELVES FOLDED UNDER
END OF SHELVES BENT DOWN AND RIVITED TO SIDE
COMMON TRUNK LATCHES

FIG. 1

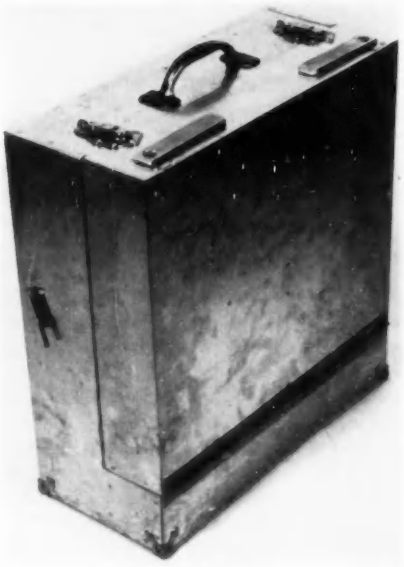

FIG. 2

FIG. 3

able so that either two racks, two trays, or one of each may be carried. Various dividers may be used in the drawer according to the use planned for the kit. The rack is designed to hold 56 standard, 15 ml., conical, centrifuge tubes and is provided with a cork base to resist breakage (Fig. 1).

A wide variety of equipment may be compactly arranged in the kit to satisfy the particular needs of the field worker. Fig 3 shows the type of equipment which may be carried on one field trip. All of the equipment shown fits compactly into the kit.

The kit may be used along the side of a road, on any flat surface in the field, or on the tailgate

FIG. 4

of a jeep or other vehicle. A carrying strap is provided which hooks on to the sides of the kit and facilitates carrying it for long distances, through brush, or over rough terrain (Fig. 4).

The kit has been used mainly for collecting blood and serum, preparing blood smears, collecting arthropod vectors, and dissecting small mammals. By changing the equipment carried in the kit it may be adapted for other field uses.

The value of a kit such as the one described is certainly dependent upon the individual investigator. Its adaptability, light weight, compactness, strength, and durability make it excellent for many field studies and in many investigations it aids in planning as well as in handling equipment needed in the field.

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INFECTIVITY TO MOSQUITOES OF *PLASMODIUM VIVAX* FOLLOWING TREATMENT WITH CHLOROQUINE AND OTHER ANTIMALARIALS

G. M. JEFFERY

Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Tropical Diseases, P. O. Box 717, Columbia, South Carolina

In an area where malaria eradication is an objective the selection of an acceptable antimalarial drug is dependent not only on the basis of rapid effect on clinical malaria, but also on its ability to halt mosquito transmission with rapidity. Numerous studies have been done to show the rapid reduction of parasitemias and the rapid elimination of clinical malaria after various drugs, but few have elucidated the actual cessation of infectivity to mosquitoes of treated cases of malaria. Recent studies from this laboratory have described the sporontocidal effects of various drugs upon *Plasmodium falciparum* (Ramakrishnan *et al.*, 1952; Jeffery *et al.*, 1956). The present report is concerned with the ability of several drugs to interrupt the sporogonous cycle of *P. vivax*.

METHODS

White patients undergoing malaria therapy for neurosyphilis provided the subjects. The malaria used was the Chesson strain of *P. vivax* induced either by the bites of infected mosquitoes (*Anopheles quadrimaculatus*) or by transfusion of 10 to 20 cc. of infected blood.

Total parasite densities were determined on Giemsa-stained Earle-Perez type thick films made daily or more often. Gametocytes were enumerated on thin films in terms of gametocytes per number of white cells.

A. quadrimaculatus were fed on patients prior to administration of drug and at various times following, depending on the schedule of the particular experiment. Mosquitoes were maintained in an insectary at 78° F. during the incubation period. Generally about 25 mosquitoes from each lot were dissected 12 ± 1 days after feeding and the oocysts on the midgut enumerated. When it was desired to know if sporozoites had developed in a group of infected mosquitoes, gland dissections were done 15 to 20 days after feeding.

Drugs were administered for two purposes: for temporary suppression or modification of the clinical course or for termination of the attack. In the cases under consideration temporary modification of the attack was by means of 0.1 gram of sodium bismuth thioglycollate intramuscularly. Termination of the attack was achieved by oral chloroquine (1.5 grams base given over a 3-day period), intramuscular chloroquine (0.4 gram base, single dose), or chlorguanide (0.05 to 0.4 gram orally in 1 to 4 days). Also included are several experimental treatments with varying doses of pyrimethamine. All cases treated were primary attacks.

RESULTS

Chloroquine. Mosquito feedings were done before and at 24-hour intervals for one to three days after treatment with chloroquine in 20 cases. Mosquitoes were always fed on the first day after treatment regardless of parasite density or presence; on subsequent days feedings usually were done only when the parasite density exceeded an arbitrary limit of 50 per cmm., since extensive experience in these laboratories indicate that parasitemias of *P. vivax* at least twice as great do not produce infections in mosquitoes. Table 1 summarizes the infectivity after chloroquine. In the 15 cases where mosquito feedings were continued to the last parasite day, or until none became infected, seven were noninfective to mosquitoes within 24 hours after treatment. Infectivity persisted for one day in eight cases and as long as two days in only a single case. In the five cases where mosquito feedings were halted when the parasitemia descended to 50 per cmm. or less, infectivity persisted for one day in all and for two days in a single case. In the cases where the infectivity persisted through the first and second post-treatment days the infections were usually of a lower grade than prior to treatment. Gland dissections of mosquito lots showing gut infections revealed normal-appearing sporozoites.

TABLE 1

Effect of chloroquine on the infectivity to mosquitoes of P. vivax *(Chesson strain)*

Patient number	Chloroquine regimen	Days after first patency drug given	Total parasites per cmm. on day of treatment	Gametocytes per 200 wbc on day of treatment	Days after treatment last parasite seen	Infectivity to mosquitoes (% inf.)				
						Day of treatment (D)	D + 1	D + 2	D + 3	D + 4
Mosquito feedings continued to elimination of parasites or infectivity:										
G 404	1.5 g. oral	30	28,800	151	3	83.3	56.0	0.0	0.0	*
G 422	1.5 g. oral	17	4,968	3	3	100.0	87.5	0.0	0.0	*
G 406	1.5 g. oral	25	14,688	117	3	100.0	88.2	4.0	0.0	*
G 213	1.5 g. oral	16	13,800	21	4	77.7	83.3	0.0	**	**
G 459	1.5 g. oral	22	20,160	7	2	76.9	66.6	0.0	*	*
G 461[a]	1.5 g. oral	49	6,096	1	1	100.0	0.0	0.0	*	*
G 424	0.4 g. i.m.	6	32,880	2	3	100.0	100.0	0.0	**	*
G 298	0.4 g. i.m.	32	4,848	7	1	80.0	0.0	*	*	*
G 353	0.4 g. i.m.	24	7,776	7	1	11.5	0.0	*	*	*
G 326	1.5 g. oral	29	9,800	18	1	100.0	0.0	*	*	*
G 330	1.5 g. oral	21	4,290	16	1	60.0	2.9	*	*	*
G 332	1.5 g. oral	27	7,920	5	1	88.9	40.0	*	*	*
G 336	1.5 g. oral	12	11,070	5	4	33.3	0.0	**	*	**
G 325	1.5 g. oral	22	4,480	4	0	69.2	0.0	*	*	*
G 374	1.5 g. oral	44	3,144	1	1	24.0	0.0	*	*	*
Mosquito feedings continued to parasite density of 50 per cmm. or less:										
G 401	0.4 g. i.m.	25	22,600	73	4	87.5	80.0	84.0	**	**
G 392	0.4 g. i.m.	14	13,050	1	3	30.8	3.9	**	**	*
G 319	1.5 g. oral	23	6,720	18	2	100.0	80.0	**	*	*
G 396	1.5 g. oral	21	25,920	17	2	35.3	44.0	**	*	*
G 461	1.5 g. oral	27	7,020	8	2	100.0	32.0	**	*	*
Chloroquine + primaquine (30 mg./day × 3 days)										
G 400	1.5 g. oral	25	2,560	10	2	93.3	0.0	**	*	*

* = No parasites present
** = Parasites 50/cmm. or less } —No mosquito feedings.
[a] = Second inoculation.

In a single case where primaquine (30 mg. per day × 3) was added to the oral chloroquine regimen, parasites were eliminated after two days, and infectivity to mosquitoes within 24 hours.

Chlorguanide. Mosquito feedings were done on four cases treated with chlorguanide. One of these cases was treated with a single dose of 50 mg., one with a single 100-mg. dose, and two with four consecutive daily doses of 100 mg. In all cases infectivity to mosquitoes ceased 24 hours after treatment. However, 48 hours after the single 50-mg. dose infectivity to mosquitoes returned and persisted through the fifth post-treatment day, after which the parasite density descended to about 100 per cmm.; parasites were absent after day seven. Sporozoites developed normally in these mosquitoes and an attempted transmission was successful. In the cases given 100 mg., either in a single dose or the dose repeated daily for four days, infectivity to mosquitoes did not return and the parasites were removed after three to seven days; gametocytes persisted for two to four days. In each case the drug aborted the primary attack.

Pyrimethamine. A case of Chesson *vivax* treated with 25 mg. of pyrimethamine became

TABLE 2

Gametocyte densities and mosquito infections before and after treatment of 10 cases of P. vivax *(Chesson) with 0.1 gram sodium bismuth thioglycollate, with comparable data on 7 untreated controls*

Patient No.	Day of Treatment (D)*		D + 1		D + 2		D + 3		D + 4	
	Gamet. per 200 wbc	% mosq. inf.	Gamet. per 200 wbc	% mosq. inf.	Gamet. per 200 wbc	% mosq. inf.	Gamet. per 200 wbc	% mosq. inf.	Gamet. per 200 wbc	% mosq. inf.
Treated cases										
G 326	3	52.0	0	0.0	1	0.0	4	25.0	1	0.0
G 319	2	23.1	0	0.0	1	0.0	0	85.3	1	4.3
G 312	5	66.7	0	0.0	1	91.7	16	100.0	5	14.8
G 302	2	9.8	0	0.0	1	0.0	16	75.0	6	**
G 297	3	2.9	1	84.0	1	0.0	4	100.0	5	0.0
G 276	2	80.6	0	0.0	2	74.2	6	90.5	3	48.6
G 406	1	100.0	0	0.0	0	89.7	3	84.2	1	60.0
G 407	1	60.0	0	0.0	1	100.0	1	100.0	7	**
G 401	5	47.1	0	0.0	1	0.0	11	100.0	7	93.5
G 412	3	83.3	0	0.0	1	0.0	2	83.3	1	0.0
Untreated cases										
G 244	6	100.0	3	80.0	5	100.0	**	**	**	**
G 190	2	14.3	12	0.0	6	14.3	4	0.0	10	0.0
G 252	2	95.0	3	100.0	2	37.5	9	14.7	5	10.0
G 87	7	100.0	15	100.0	7	8.3	0	0.0	0	0.0
G 86	1	100.0	6	26.7	9	75.0	6	80.0	20	94.3
G 66	8	14.0	1	5.7	2	0.0	1	43.9	5	100.0
G 171	11	100.0	3	73.3	7	100.0	5	100.0	3	**

* = In untreated cases "Day of treatment" was set at day 8 of patent parasites, since this was the usual day of treatment in the treated group.

** = No mosquito feedings or no parasite data.

noninfective to mosquitoes within four hours post-treatment and subsequent feedings at 8, 12, and 24 hours showed no viable oocysts, although gametocytes and asexual forms persisted in the peripheral blood for six and eight days. However, such was not the case where a pyrimethamine-resistant strain was concerned. In an infection made pyrimethamine-resistant by stepwise increases of noncurative dosages (case AP-309 of Hernandez *et al.*, 1953) mosquitoes were fed on the sixth day of a seven-day course of 25 mg. of drug daily; further feedings were done two and four days after cessation of the 25-mg. dosages. In all cases these mosquitoes became infected, developed normal sporozoites and were capable of passaging the infection.

Sodium bismuth thioglycollate. Although not generally useful as an antimalarial this drug has been used to a considerable extent in the management of therapeutic malaria, especially in *P. vivax* (Young *et al.*, 1943). The effect of this drug on the infectivity of the malarial infection to mosquitoes has long been noted. In over 25 cases feedings were done one day after administration of 0.1 gram of the drug intramuscularly and in only one was it possible to infect mosquitoes. Although the drug generally has little effect on the total parasite density, almost invariably gametocytes disappear from the peripheral blood and are absent through the first post-treatment day. Table 2 summarizes mosquito feedings done before and after treatment in 10 cases and also presents similar data for seven comparable cases where no drug was given. The drug was administered usually on the eighth day of parasites, with a mean of 7.8 days; therefore, in the controls the eighth day of parasites was arbitrarily considered the "day of treatment." Infectivity to mosquitoes had returned in only four of the ten treated cases two days after treatment, but was again present in all at three days. The normal variability of

this strain in gametocytemia and in infectivity to mosquitoes is demonstrated in the seven untreated cases.

DISCUSSION

It is apparent that in this strain of *P. vivax* the rapid effect of chloroquine on the asexual parasites, as reported by Jeffery (1956), is paralleled by its rapid removal of the sexual forms. This is in contrast to the situation which exists in *P. falciparum* (Jeffery *et al.*, 1956) where the asexual forms were rapidly reduced but gametocytes persisted for 15 to 26 days and infectivity to mosquitoes as long as 20 days post-treatment. Thus it appears that the addition to the chloroquine regimen of a gametocytocidal drug such as primaquine, necessary for rapid elimination of viable gametocytes of *P. falciparum*, would not be of importance in *P. vivax*. In the only case tried, such an additional drug eliminated infectivity as early as 24 hours after treatment, as did chloroquine alone in seven of 15 cases, but in neither case were earlier feedings done to determine earlier elimination.

The results of treatment with chlorguanide and pyrimethamine suggest that sufficient dosages are effective in rapid termination of the malaria attack and elimination of infectivity to mosquitoes, but that with lower dosages of chlorguanide the infectivity may return. However, where the *vivax* strain has become resistant to pyrimethamine the resistance is also operative where the infectivity to mosquitoes is concerned. Multiple dosages of drug which ordinarily would eliminate the parasites and infectivity within 24 to 48 hours, failed to affect materially either the parasitemia or infectivity where drug resistance had been produced in the strain. Experience with chlorguanide against *P. falciparum* (Ramakrishnan *et al.*, 1952) has shown that the elimination of infectivity to mosquitoes after single doses of 100 and 300 mg. is only temporary; gametocytes persist in large numbers and again become infective after only a few days. Pyrimethamine (25 and 125 mg.) used against *P. falciparum* (Jeffery *et al.*, 1956) indicates that viability of gametocytes is removed very rapidly, even though gametocytes persist until the return of asexual forms.

Although of little practical interest other than in the management of therapeutic malaria, the effect of sodium bismuth thioglycollate on infectivity is suggestive of an interesting selective action by this drug. Young *et al.*, (1943) found this drug to be most effective against half-grown asexual *vivax* parasites, with little effect on parasites either younger or older than 16 to 28 hours. The reason for activity against either this range of asexual parasite or the development of gametocytes is not apparent. In contrast to the action of pyrimethamine or chlorguanide the gametocytes are usually removed from the peripheral blood in 24 hours, although it is not known whether this is by destruction of those already in the blood or by prevention of development of a fresh supply, or both. Equally perplexing is the rapid return of gametocytes to normal numbers in the peripheral blood at 48 and 72 hours post-treatment; indeed the gametocyte density often appears to be higher than normal three and four days after administration of the drug.

SUMMARY

In 20 cases of Chesson strain *Plasmodium vivax* malaria treated with chloroquine, infectivity to mosquitoes ceased as a rule after the first post-treatment day; in one case only, after the second. In three cases treated with at least 100 mg. of chlorguanide, infectivity to mosquitoes was terminated within the first 24 hours. In one instance a single 50 mg. dose of chlorguanide interrupted infectivity 24 hours, but infectivity then returned and persisted as long as sufficient parasites were present. In a single case treated with 25 mg. of pyrimethamine, infectivity was eliminated within four hours. However, a case resistant to this drug readily infected mosquitoes during and after multiple daily doses. Intramuscular sodium bismuth thioglycollate (0.1 gm.) temporarily eliminated gametocytes and infectivity to mosquitoes within 24 to 48 hours, but both reappeared after one or two days.

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THE TREATMENT OF ENTEROBIASIS IN CHILDREN. A COMPARATIVE STUDY OF PIPERAZINE (ANTEPAR) AND PYRVINIUM CHLORIDE (VANQUIN)

THOMAS S. BUMBALO, LOIS J. PLUMMER, AND J. ROGER WARNER

Department of Pediatrics, School of Medicine, University of Buffalo, and Edward J. Meyer Memorial Hospital, Buffalo, New York

Even though many studies have been reported on the successful treatment of pinworm infection with piperazine, the incidence of infected children in some communities remains relatively high and constant (Birch et al., 1957; Bumbalo et al., 1957). Approximately 30 per cent of the general pediatric population in the Buffalo, N.Y., area are infected with pinworms. The rate of infection in orphanages in this same area is about double that in the noninstitutionalized pediatric population. While wide dissemination of pinworm eggs in the environment, with constant hazard of reinfection and retroinfection, is an important factor in the high incidence of enterobiasis, the authors believe that further simplification of treatment regimes can aid in decreasing the incidence of this infection. To date, we have evaluated: Egressin, Diphenan, gentian violet, oxytetracycline, papain, cremothalidine, carbomycin, garlic, piperazine, promethazine hydrochloride and pyrathiazine. The results obtained with the first nine drugs have been published by the authors (Bumbalo, et al., 1957; Bumbalo, et al., 1954). While excellent results have been reported in the treatment of enterobiasis with promethazine hydrochloride by Avery (1956), the authors obtained a cure rate of only 5.3 per cent in a group of 107 children treated with this drug, and a cure rate of 12.5 per cent in a group of 24 children treated with a closely allied drug, pyrathiazine (Pyrrolazote) (Bumbalo, et al., 1957).

The object of this study was to compare the efficacy of pyrvinium chloride, a comparatively new oxyuricide, with that of piperazine, a well-proved drug, given in full doses in shorter courses of treatment.

CLINICAL MATERIAL AND METHODS

All of the children at the Immaculate Heart of Mary Home, an out-patient project of the Edward J. Meyer Memorial Hospital in Buffalo, New York, were included in this study. Of the 195 children, 133 (71%) were infected with pinworms. The children ranged in age from 5 to 16 years. Of these children 119 were boys and 76 were girls. Of the 195 children 167 were white, 12 were Negro, 8 were Puerto Rican and 8 were American Indians.

The diagnosis in all cases was established by the use of a modification of the Graham Scotch-tape-swab technique, which we have described in detail in an earlier publication. Two diagnostic smears were taken on two consecutive mornings before the children were bathed. Seven days after treatment, one Scotch-tape-swab smear was taken, before the children were bathed, on seven consecutive mornings to determine the results of the treatment. Those patients whose smears were negative on these seven consecutive days were declared cured.

In addition to the 139 infected children, the remaining 56 noninfected children were treated in order to minimize the possibility of reinfection by allowing infected children, who at that time may have had negative diagnostic smears, to go untreated and possibly reinfect the treated children. The total number of patients were divided into four groups, comparable as to age and sex. Table 1 indicates the number of children treated in each of the four groups, the dosage of the drugs, the days treated and the per cent of cures with each drug. Group I and II were treated with piperazine citrate (Antepar) and Groups III and IV were treated with pyrvinium chloride (Vanquin).

RESULTS AND DISCUSSION

The efficiency and lack of toxicity of piperazine as an oxyuricide has been adequately established. Cure rates ranging from 84 per cent to 100 per cent have been reported in studies from many parts of the world (Bumbalo, et al., 1957). In a recent study we treated 37

TABLE 1

Groups	Cases	Daily dosage	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.	% cured
I	34	3 gm, once	—	—	—	A	A	A	90.6
II	34	3 gm, once	A†	—	—	—	—	A	86.2
III	35	1.5 mg/kg*	V†	V	V	V	V	V	100
IV	36	2.1 mg/kg*	V	V	V	V	V	V	97.1

* In two equal doses.
† A = Antepar; V = Vanquin.

infected children with 70 mg of piperazine per pound of body weight, with a maximum of 3 gm in one, single dose. While the cure rate (43.2%) was low, the lack of toxic or untoward reactions suggested a repeat study using the maximum single dose of 3 gm once daily regardless of age and weight for 3 consecutive days (Group I) and another group (Group II) for 2 days with a 4-day rest period between the 2 treatment days. The piperazine used in this study was the syrup of Antepar containing piperazine citrate, equivalent to 100 mg of piperazine hexahydrate per ml. Each patient in Groups I and II received 6 teaspoons of the syrup (3 gm of piperazine citrate) taken in one, single dose. All of the patients in these two groups took the syrup readily and tolerated it very well. No toxic or untoward reactions were encountered in this group of children treated with a single, large dose of piperazine. The cure rate in Group I was 90.6 per cent and in Group II 86.2 per cent.

Royer (1956) was the first to report on the use of pyrvinium chloride in the treatment of pinworm infection in children. Sawitz and Karpinski (1956) have also recently reported on the successful treatment of human enterobiasis with this drug. Pyrvinium chloride is a cyanine dye known as 6-dimethyl-amino-2-[2-(2,5-dimethyl-1-phenyl-3-pyrryl) vinyl]-1-methylquinolinium chloride dehydrate. The above-named authors also have reported favorably on the acute and chronic toxicity studies in animals and the high efficacy of this drug in treating mice and rats infected with *Syphacia obvelata* and *Aspiculuris tetraptera*.

In this study we have treated 71 children, infected with pinworms, with pyrvinium chloride. The children in Group III were treated with 1.5 mg of pyrvinium chloride per kg divided into two equal doses, daily for 6 consecutive days; those in Group IV received 2.1 mg of the drug per kg divided into two equal doses daily for 6 consecutive days. The drug was given orally as an aqueous suspension flavored with raspberry syrup. All of the children accepted and tolerated the syrup very well, and no instances of gastrointestinal irritation or any other unfavorable reactions were experienced by these patients. The cure rate of the patients studied in Group III was 100 per cent and, in Group IV, 97.1 per cent. The cure rates in these groups compare favorably with the reports of Royer (1956) and of Sawitz and Karpinski (1956) and confirm the 100 per cent cure rates reported by these investigators.

None of the patients treated in this study with either piperazine or pyrvinium chloride received any previous preparation, post-treatment enemas or cathartics. Except for the usual hygienic measures of the average orphanage, no special measures of cleanliness or hygiene were practicied in treating these children.

The 56 noninfected children that were also treated in comparable groups with either piperazine or pyrvinium chloride remained negative following the same treatment that was given to the infected children. As in the infected groups, no remarkable reaction was obtained in this group of children. Sample post-treatment blood counts and urinalyses done on ten of the patients treated with piperazine and ten of the patients treated with pyrvinium chloride were normal.

The cure rates obtained in this study with pyrvinium chloride were higher than those obtained with piperazine. However, in Groups I and II the patients were treated with piperazine for 3 days and 2 days, respectively, while all of the patients treated with pyrvinium chloride were treated for 6 consecutive days. Since 100 per cent cure rates have also been reported in the treatment of enterobiasis with piperazine (Bumbalo, et al., 1957; Ferguson, et al., 1955), the authors are of the opinion that if therapy with piperazine was continued for a total of 6 days, the results would very likely compare favorably with the

results obtained with pyrvinium chloride. Since the object of this study was to determine the cure rate of shorter and simpler courses of treatment, piperazine, which has had adequate clinical trial, was selected for the short, single-dose regimen. Indeed, a cure rate of 90.6 per cent in Group I and 86.2 per cent in Group II indicates that piperazine still is a drug of choice in clinical practice and in such situations where more prolonged courses of treatment may not be practical or possible.

SUMMARY

Results of treatment of enterobiasis in children with two efficient oxyuricides are reported. The percentage of cures in a group of 34 infected children treated with a single daily dose of 3 gm of piperazine for 3 days was 90.6 per cent. The cure rate among 34 infected children treated with a single daily dose of 3 gm of piperazine for 2 days was 86.2 per cent. The cure rate among 35 infected children treated with 1.5 mg/kg of pyrvinium chloride daily for 6 days was 100 per cent. The cure rate among 36 infected children treated with 2.1 mg/kg of pyrvinium chloride daily for 6 days was 97.1 per cent.

Both piperazine and pyrvinium chloride were well accepted and tolerated, and no untoward or toxic reactions were experienced by any of the children.

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MAN-BITING MOSQUITOES IN COASTAL LIBERIA

RICHARD M. FOX

The Liberian Institute of the American Foundation for Tropical Medicine, Harbel, Liberia, West Africa[1]

The first systematic review of the mosquitoes of Liberia was presented by Bequaert (1930). Additional information was collected by Barber, Rice and Brown (1932), Young and Johnson (1949), Briscoe (1947, 1950, 1952), and Gelfand (1955a, 1955b), largely as outgrowths of studies in mosquito-borne diseases. Peters (1955, 1956) made an extensive study of the Liberian culicine fauna, recording 81 species of which three were new and 41 were recorded from Liberia for the first time. Particular attention naturally has centered on three or four species of major medical importance and scant data are available on adult food habits of other culicines.

Aimed primarily at a study of malaria transmission in this area, a program of nightly collections of *Anopheles gambiae* using human bait was begun at the Institute in mid-December, 1954. During the course of this work a number of other species were collected. Since many of these other mosquitoes have been shown to be disease vectors and the others, being man-biters, must be suspected, the information accumulated is presented herewith.

Three methods for collecting anthropophilic mosquitoes were used: a portable trap was occupied by a collector from sunset to sunrise; a team of two collectors, hand-caught, alive, the mosquitoes attempting to bite them as they sat in the open; mosquitoes found resting in native houses were hand-collected alive in the morning. For the first six months these nightly collections were made in work camps on the Firestone Rubber Plantation. In June, 1955, operations were shifted to Charlesville Township, Marshall Territory, in the center of which the Institute is situated, where collections were made in native villages. For four months in the spring of 1955 collections were made once a week beside a permanent swamp located at some distance from human habitation, using only the night teams and the trap. During the dry season of 1956–1957 operations were concentrated on two small villages near the Institute, using two night teams collecting every night.

During September and October, 1955, a Jersey light trap was operated on the Institute grounds. This produced several anophelines not collected in anthropophilic situations here but said to be malaria vectors elsewhere. For that reason these records are included below.

The ecologic divisions in Liberia are discussed by Briscoe (1952). Stations employed in the present study can be divided into four ecologic groups: (1) Firestone Plantation labor camps are located in a hilly area of rising land from 15 to 50 miles back from the coast. It is densely planted in rubber; about 25 per cent of the surface is swampy, unsuited for rubber cultivation and, in general, grows wild. A few tracts of the original, primary, rain forest remain untouched within the boundaries of the plantation. (2) Charlesville Township is rather flat, lies between the southern boundary of the plantation and the Gboe and Farmington rivers and includes the Institute and Robertsfield air port. Originally rain forest, this area has been under sporadic native cultivation for many years and is largely covered with low, second-growth bush. In the dry season about 25 per cent of the area is boggy or swampy, but during the rains the proportion is very much higher. (3) Along the southern (seaward) side of Charlesville Township there is a narrow strip of coastal savannah. This is sandy soil, supporting grass meadows and is laced with galleries of oil palm and dense thicket growing along little streams. During the rainy season much of this is under water. Sammy village was the only station in the coastal savannah. (4) The lower reaches of the Gboe and Farmington rivers are bordered with mangrove swamps. Snafu and Cowfarm villages are on pieces of higher ground in the mangrove.

Rainfall recorded at Robertsfield averaged 160 inches annually for the past seven years. To the south of the air field and nearer the coast the rainfall is higher; records kept at the Institute for 1956 and part of 1957 indicate that

[1] Present address: Carnegie Museum, Pittsburgh 13, Pennsylvania, U. S. A.

about 10 per cent more rain falls at the Institute than at the air field. Records kept on the plantation show that rainfall decreases as one goes inland. The rainy season begins in May and continues into October with particularly massive downpours in June and September. October to May is the so-called dry season during which any one month normally has only from one to ten inches of rain. Temperatures range from 60° to 89° in the shade; humidity is generally higher than 90 per cent. A more detailed discussion on climate related to mosquitoes was presented by Fox (1957).

Identifications of most of the mosquitoes listed below were made from Edwards (1941) and de Meillon (1947). I am indebted to Mr. P. F. Mattingly of the British Museum (Natural History), who kindly checked and corrected my identifications of some of the more difficult and unusual species.

Anopheles ziemanni Grünberg, 1902. From late January until early March 1957 this species was regularly taken while attacking the night collectors, but never more than a few individuals on any one night. Previously, one female had been found resting in a hut in Kakata, a town situated a short distance north of the Firestone Plantation, in June. Two more had been found in July near the Institute, one of these entering the man-baited trap. It appears to be locally common in the hinterland, according to records of the W.H.O., Malaria Control Project at Kpain. In Uganda *A. ziemanni* was found to be attracted to goats. In this area there are very few goats, but apparently it does not hesitate to bite man.

Dissections failed to implicate this species locally as a vector of either malaria or filariasis. No positives were found in 20 midgut dissections, 22 salivary gland dissections or 22 teased dissections for filariae.

In some parts of its range *A. ziemanni* is sympatric with *A. coustani*, of which the former is usually listed as a subspecies. From this it should be clear that *A. ziemanni* cannot be a subspecies of *A. coustani*, and can be either a valid and separate species or an infraspecific genetic variation, in which case the name *ziemanni* is not needed.

Anopheles paludis Theobald, 1900. This dry-season mosquito appears in this area in December and persists until June or July, depending upon how soon the heavy rains begin. All specimens taken, both in Charlesville and on the Firestone Plantation, were captured either by night teams or in the man-baited trap; none was found resting in huts. It is most plentiful during January, February and March while the *A. gambiae* population is at its lowest. It was not found in savannah or mangrove areas. It appears to be anthropophilic and strongly exophilic. An attempt was made to colonize the species in our laboratory but the third generation of adults failed to produce fertile eggs. It was observed that the adults live much longer in the laboratory than does *A. gambiae* and that larval development is slower.

Dissections were made on 29 midguts and 28 salivary glands; all were negative for malaria. Of 25 teased dissections made for filariae, one contained two second-stage larvae in the thorax, about 325 μ long, but these could not be identified positively as being bancroftian.

Anopheles barbarellus Evans, 1932. The light trap caught one female in September and one in October. A third female was found by a night team in April. The type locality is the Firestone Plantation. It appears not to be a man-biting species.

Anopheles funestus Giles, 1900. A night team collected one female on the Firestone Plantation in February and another was taken in the light trap in October at the Institute. A major malaria vector in much of Africa and quite common in the hinterland plateau of Liberia, it is exceedingly rare in the area studied. Since *A. funestus*, a strongly endophilic species, is readily eradicated by residual sprays, its absence from the Firestone Plantation doubtless is the result of the spraying there and evidently the U. S. Army eliminated it from the Charlesville area during the last years of World War II.

Anopheles hargreavesi Evans, 1927. Thirteen females were taken in the light trap in September and October 1955. Since none was captured during the extensive program of collecting for man-biting species, it is concluded that it is not anthropophilic here, although it is believed to be of importance as a malaria vector in southern Nigeria (de Meillon, 1947).

Anopheles hancocki Edwards, 1929. Eighty six females were collected, two during the rains and all the rest during the dry season from late September until March. This species was taken by all collecting methods, including the light trap, but only one was found resting in a hut. The species bites man, quite possibly other

animals as well. In the area studied the population peak occurs in October, November and December.

Twenty six dissections of midguts and 28 salivary gland dissections made were negative for malaria. Twenty nine dissections for filariae also were negative. In Uganda the species was found to have natural malaria infections.

Anopheles gambiae Giles, 1902. This species is common throughout the year in all parts of the area studied. In the vicinity of the coastal strip of mangrove marsh it shares with *A. melas* the dubious distinction of being the predominant man-biting mosquito, while in Charlesville and on the Firestone Plantation it makes up more than 99.5 per cent of the anophelines captured in anthropophilic situations. Found in substantial numbers at all times, the population reaches its peak during the rainy season from June through September. It is catholic in its food tastes (Fox and Fox, 1955), and we have taken it using a trap baited with chimpanzee. In the laboratory *A. gambiae* feeds readily on man, on chimpanzee and on rabbit, accepting any blood meal offered and quite willingly varies the diet on successive feedings. On one occasion it was induced to feed on a snake, although this is certainly not a preferred source of a blood meal for *A. gambiae* and probably never occurs in nature. It illustrates the lack of compulsory food choice in the species. It bites freely outdoors and does not need to enter houses to feed, though it does not hesitate to do so. Larvae have been collected in this vicinity in all manner of water deposits, both temporary and permanent. A more detailed account of this insect and its local role in disease transmission has been presented elsewhere (Fox, 1947).

Anopheles melas Theobald, 1903. Gelfand (1955b) discussed *A. melas* in this area. Here, as elsewhere, it normally develops in the brackish pools located between daily high-tide level and semimonthly spring-tide level in the *Avicennia* mangrove swamps. That brackish water is not an essential physiologic requirement is indicated by the fact that we collected larvae in season and in abundance from rain water accumulated in the hulk of an old dugout canoe partly sunk in the muck of a *Rhizophora* mangrove swamp. Our laboratory colony does very well in distilled water and after a few generations loses its tolerance for salt water. The species flies from December to July and disappears almost completely with the onset of the heavy rains, probably as the result of the fact that the rise in mean level of the tidal rivers inundates the usual breeding pools. Breeding in the laboratory colony continues actively through the rainy season, but in nature only a very few estivating adults are to be found.

TABLE 1

Month	*A. gambiae*			*A. melas*		
	Number	Per cent	Per collection	Number	Per cent	Per collection
Nov., 1955	34	100.0	4.9	0	0	0
Dec., 1955	13	86.6	1.5	2	13.4	0.2
Jan., 1956	13	39.5	2.2	20	60.5	3.3
Feb., 1956	32	36.8	2.7	55	63.2	4.6
Mar., 1956	12	30.0	3.0	28	70.0	7.0
Apr., 1956	55	26.2	7.9	155	73.8	22.2
May, 1956	28	26.7	4.7	77	73.3	12.9
June, 1956	58	62.3	8.3	35	37.7	5.0

Our own records of the population of *A. melas* and its relation to the *A. gambiae* population in the same place are based on weekly collections of females resting in huts at Cowfarm, a village located on a peninsula of higher ground surrounded by mangrove swamps near the confluence of the Farmington and Gboe rivers. These data are summarized in Table 1 and agree with Gelfand's findings (1955b).

These data should be taken only as a rough indication of the true proportion between the two species at Cowfarm, since they include, of necessity, only those females laying eggs and thus permitting identification. There is no way of knowing whether both species lay eggs at the same rate or what the identifications might have been of those not producing eggs.

The systematic position of *A. melas* is uncertain. Because the adults cannot be distinguished from those of *A. gambiae*, students of culicine taxonomy frequently designate *A. melas* as a "variation" of *A. gambiae*. This is a convenient way to beg the question and represents a convention useful under the circumstances. The word "variation" has no real meaning in taxonomy (Fox and Fox, 1955). Gelfand (1955b) and others have treated *A. melas* as a valid species. Certainly *A. melas* and *A. gambiae* are sympatric in part. In consequence they may be different species or they may be identical, but they cannot be conspecific subspecies. The two

applications of the breeding test made in the past (Muirhead-Thomson, 1948; Bruce-Chwatt, 1950) produced conflicting results.

Mansonia (*Coquilletidia*) *cristata* (Theobald), 1901. A female was taken at Kakata in June and another was found on the Firestone Plantation in December, both by night teams.

Mansonia (*Mansonioides*) *uniformis* (Theobald), 1901. This and the next species have been implicated as vectors of filariasis in certain parts of the world, but Gelfand (1955a) did not find mature larvae either in a survey of wild females or during the course of laboratory tests. *M. uniformis* is widely distributed in tropical Africa and in India. In coastal Liberia it was found to be a dry-season species attacking man voraciously and was not infrequently discovered resting in native houses. It begins to appear in October as the rains taper off, is common from January to April, reaching a population peak in February, and disappears during May as the rainy season begins. We never found it from June to September.

Mansonia (*Mansonioides*) *africana* (Theobald), 1901. This species is much more limited in distribution than the preceding. In coastal Liberia it is not nearly so common. It, too, is a dry-season species, but the population peak is reached in late November and early December; it begins to disappear during March.

Aedes (*Finlaya*) *longipalpus* (Grünberg), 1905. A single female was taken in the man-baited trap in Charlesville in June and one larva was found in a tree hole in November.

Aedes (*Stegomyia*) *aegypti* (Linné), 1762. This species was found throughout the year in every part of the area. Adults are commoner during the dry season. Equal numbers were taken in the trap and by night teams; a few were found resting in houses. For some months collectors were sent out by day from time to time and stationed in shady underbrush for some hours at a time; *A. aegypti* was the only species found biting. It was one of the species collected in a trap baited with chimpanzee. Larvae were found in every month in permanent pools, in wells, in rain puddles, in tree holes, in tin cans and utensils. On the rubber plantation, when latex cups are not inverted while not in use, rain water collects in them and quickly becomes infested with *A. aegypti* larvae.

Aedes (*Stegomyia*) *africanus* (Theobald), 1901. Like the foregoing species, this is found throughout the year. The population peak occurs in April and the species is commonest from March through May, but seems never to be as common as *A. aegypti*, at least in anthropophilic situations. A few were found resting in houses, but the majority were taken in equal numbers by night teams and the man-baited trap. Apparently it feeds readily indoors but does not usually remain there to rest. It, too, was taken in the trap baited with chimpanzee.

Aedes (*Stegomyia*) *apicoargenteus* (Theobald), 1910. On the Firestone Plantation a female was taken in the man-baited trap in March, another by a night team in November. Larvae were found in a well in Charlesville in October. A female was taken in November resting in a native hut.

Aedes (*Stegomyia*) *vittatus* (Bigot), 1861. A single female was collected by a night team in Charlesville in May.

Aedes (*Aedimorphus*) *argenteopunctatus* (Theobald), 1901. Larvae were found in a puddle near the Institute in November, developing with *Culex decens*. In Charlesville night teams collected five females from the end of February to the middle of April. Edwards (1941) records the species from Sierra Leone and the Gold Coast; Peters (1956) did not find it. Evidently this is the first record for Liberia.

Aedes (*Banksinella*) *fuscinervis* (Edwards), 1914. A single female was collected by a night team in Charlesville in April.

Culex (*Lutzia*) *tigripes* Grandpré and Charmoy, 1900. Five females were collected on the Firestone Plantation in February to May. Two of these were taken by night teams; three in the man-baited trap. One female was found resting in one of the staff houses at the Institute in October. It would appear to be a dry-season species.

Culex (*Neoculex*) *galliardi* Edwards, 1941. One female was taken in the man-baited trap in November in Charlesville. The species was recorded from Nigeria and Sierra Leone by Edwards (1941); the identification of this first Liberian record was confirmed by Mr. Mattingly.

Culex (*Culiciomyia*) *nebulosus* Theobald, 1901. Larvae were found in tree holes in September, December and February. Very few adults were taken during the rainy season and the popula-

tion peak appears to be in April. Night teams on several occasions captured males flying around the females attempting to feed. Females were sometimes found resting in native houses.

Culex (*Culiciomyia*) *cinereus* Theobald, 1901. Night teams captured three females, two on the Firestone Plantation and one at Kakata to the north; two of these were found in May, one in December.

Culex (*Culiciomyia*) *macfiei* Edwards, 1923. The man-baited trap took one female in December on the Firestone Plantation.

Culex (*Mochthogenes*) *inconspicuosus* (Theobald), 1908. Adults were taken only at night, near the Institute in November. Peters (1956) found both larvae and resting adults. Apparently this species does not feed on human blood.

Culex (*Culex*) *thallasius* Theobald, 1902. This species is said to develop in brackish water similar to that preferred by *Anopheles melas* (Peters, 1956). If this is so, *C. thallasius* must have a greater flight range than does *A. melas*. In November, night teams took it in the southern part of the Firestone Plantation. In Charlesville it was found by night teams and also taken in the man-baited trap from September to May and twice during the height of the rains in July. Nine of the 11 localities where it was found have never yielded *A. melas*. On several occasions it was found resting in native houses.

Culex (*Culex*) *pipiens fatigans* Wiedemann, 1828. We did not find this species on the Firestone Plantation and Peters (1956) does not record it from the hinterland plateau; apparently it is limited to the coastal strip. It is reported to be a common pest in Monrovia, but only six females were found in the area studied for the present report. One of these females was resting in a native house, one was taken in a man-baited trap, the other four were collected by night teams. These captures were made in October, November, January, February and March. One male larva was found in a domestic utensil in a village not far from the Institute.

Culex (*Culex*) *decens* Theobald, 1901. Peters (1956) apparently did not find this species and merely comments on Barber's (1932) records. Larvae were collected from leaf axils, temporary ground pools and domestic utensils from September to December. Adults were captured biting man in December, January and February. It does not seem to be common and may also feed on other animals.

Culex (*Culex*) *ingrami* Edwards, 1916. Larvae were found in a puddle near the Institute in December. Since females were never taken in anthropophilic situations, it is believed that this species does not feed on man.

SUMMARY

From mosquitoes collected in anthropophilic situations, 24 man-biting species are recorded with seasonal distribution and other biometric data, including six species of *Anopheles*, three of *Mansonia*, seven of *Aedes* and eight of *Culex*. *Anopheles ziemanni*, *A. paludis* and *A. hancocki* were dissected for malaria parasites in both midgut and salivary glands; all dissections were negative. Thoracic dissections for filarial larvae were also made on these three species. The only positive dissection was one *A. paludis* containing two second-stage larvae, possibly bancroftian.

Data from light trapping and larval collecting are included for four additional species, including two species of *Anopheles* and two of *Culex*. It is concluded that these four species do not feed on human blood in the area studied. *Aedes* (*Aedimorphus*) *argenteopunctatus* and *Culex* (*Neoculex*) *galliardi* are recorded from Liberia for the first time.

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YOUNG, M. D., AND JOHNSON, T. H., 1949. A malaria survey of Liberia. *J. Natl. Malaria Soc.*, **8:** 247–266.

Seasons and Weather in their Relation to Epidemics (Jahreszeit und Witterung im Seuchengeschehen)—Typhoid, Paratyphoid, Dysentery, Poliomyelitis, Diphtheria, Scarlatina. By WILHELM DONLE. 192 pp. with 18 illustrations. Stuttgart: Ferdinand Enke Verlag, 1956.

The attractively printed book by Donle may be said to fall into three parts, namely, an introduction, a main part of almost 150 pages which deals in separate sections with the six infectious diseases enumerated in the title, and a final chapter in which an attempt is made to draw general conclusions from the evidence adduced. Four and a half closely printed pages containing references are appended. Since the author deals almost exclusively with the disease situations in Central Europe, particularly in Germany, it is natural that he quotes with not many exceptions German publications.

As is pointed out in the introduction, climatic conditions may exert an influence on the occurrence of infectious diseases in various ways. They may act directly upon the causative organisms, some of which may be benefitted by cold weather while others may find particularly favorable conditions in summer, a season during which flies also may play an important role in the transmission of some of the bacteria involved. On the other hand, there can be no doubt that changes in the meteorological conditions are of great importance in bringing about increases or decreases of the susceptibility to infectious diseases, which in their turn exert a profound influence on the genesis and spread of epidemics. However, as the author maintains, so far it has not been possible clearly to demonstrate in this connexion the role of single meteorological factors like barometric pressure or moisture of the air. Generally speaking, it would appear that not any single factor, but an interplay of various factors is of importance for the appearance of both individual attacks of infectious diseases and of epidemics—a postulate with which most epidemiologists will be in agreement. Another point upon which the author insists is that factors influencing the susceptibility or resistance to infectious diseases must exert their action mainly during the incubation period which, as he states, thus "attains a quite new importance."

Recapitulating these statements in the concluding part of his study, the author stresses the importance of changes in the distribution of cold or hot air masses for the creation of favorable or unfavorable conditions for the development of epidemics. Though thus far irruptions of masses of cold air have been found to be particularly effective, changes in the opposite direction or other climatic conditions no doubt are also apt to play an important role. Thus one might follow the author in stating that, generally speaking, the genesis of diseases, particularly of epidemics, stands in connexion with the manifestation of instabilities of the weather conditions ("Durchzug von Unstetigkeitsschichten").

While pointing to the progress which has been made in the correlation of seasonal climatic changes with the incidence of epidemic diseases, the author admits that details of the action of meteorological conditions on the function of the body are still largely unknown. Moreover, in addition to the macroclimate, various microclimatic and other local environmental factors exert, no doubt, an important influence on the condition of the body in general and the susceptibility for, or the resistance to, infectious diseases in particular. Still, the already available knowledge may be said to be useful for making forecasts of the incidence of epidemic diseases and thus obtaining guidance for the conduct of preventive work. Hence, though one can not help feeling that Donle's disquisition, painstaking though it is, to a considerable extent poses questions rather than offering answers to them, it is of value in stimulating interest in a subject which is of great importance for the study and control of epidemic diseases.

R. POLLITZER

Die Geschwülste der Haut, Ihr klinisches und feingewebliches Bild, ihre Erkennung und Behandlung, VON PROF. DR. A. GREITHER UND DR. H. TRITSCH, Heidelberg [*Tumors of the Skin*, Their Clinical and Histological Picture, Diagnosis and Treatment] Stuttgart, Herdwe 63, Georg Thieme, Verlag, 1957. 280 pages, 180 illustrations; DM 49.50

The increasing importance of dermatopathology can be seen from this volume on tumors of the skin, with practically every clinical picture accompanied by a microphotograph. It is written by two dermatologists and the material is collected entirely from the Skin Department of the University of Heidelberg. The term "tumor" is used in its broadest sense and includes dermatoses which create a tumor-like picture, including, for instance, keloids, bromoderma, knuckle pads, and xanthomatoses.

The volume is well written, handsomely printed, and contains in concise form clinical description,

differential diagnosis, histological picture, and outline of treatment of the conditions discussed. Numerous clinical pictures, as well as microphotographs, are used to illustrate the text. They are generally good; some are excellent. It would have been desirable to use more high-power microphotographs. The chapter on the controversial condition of keratoacanthoma is excellent. The inclusion of Bowen's disease, Paget's disease, erythoplasia Queyrat, and Borst-Jadassohn epithelioma among precancerous conditions, such as senile keratosis and leukoplakia, has to be strongly and vehemently challenged. However, this criticism does not detract from the great value of this book, which should prove useful to anyone interested in dermatology, as well as in tumors, be he specialist or general practitioner.

PAUL FASAL

BOOKS RECEIVED

Statistical Methodology in Malaria Work, by Satya Swaroop. 246 pages, ill. World Health Organization, 1957.

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TRANSACTIONS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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MINUTES OF THE ANNUAL COUNCIL MEETING

The annual business meeting of the Council was held on October 30, 1957, with President Andrews in the Chair. All Officers and Councilors were present with the exception of M. C. Balfour and J. J. Sapero. Paul E. Thompson, Secretary of the American Society of Parasitologists, was present by invitation.

The minutes of the fifth meeting, held in New Orleans, Oct. 31–Nov. 3, 1956, were approved as published in the Journal in March, 1957.

The Secretary's report was presented as follows:

This report covers the period from October 15, 1956 to September 30, 1957. The present Secretary assumed office on January 1, 1957.

(1) *New membership list.* This was published in December 1956 by the News and, since our members have a good deal of mobility, is already partially out of date. Including new members, over 250 changes in address have been made this year.

(2) *Annual meeting.* An agreement to hold a joint meeting in 1957 with the American Society of Parasitologists was made in New Orleans. We have worked closely with the Secretary of the ASP all year in preparation therefor. Following the death of Dr. Sawitz, who had agreed to act as Local Chairman, two Co-Chairmen were appointed: Dr. C. Brooke Worth of our Society, and Dr. George L. Graham of the ASP. Announcement of the meeting was sent out in March and printed programs were mailed on September 23. The Secretary saw Dr. Worth in Philadelphia in July; otherwise all arrangements have been made by correspondence.

(3) *Membership.* As of September 30, the Society had 1,076 active members in good standing. The rolls also include 1 life member, 7 emeritus members, and 14 honorary members, making a total of 1,098 members in good standing, which is the same number as last year. In addition, there are 131 members delinquent in dues; 62 for 1957, 41 for 1956 and 1957, and 28 for 1955, 1956 and 1957, who have failed to respond to statements of dues sent out on three occasions during the year. Adding these, we have a grand total of 1,229.

Included in the above are 64 new members

elected after publication of the 1956 Transactions, whose names have been published in the NEWS.

With regret, we note the death of four honorary members:

FREDERICK G. NOVY
HENRIQUE DA ROCHA-LIMA
JEROME RODHAIN
KIYOSHI SHIGA

We also have to record the death of four active members:

SARKIS B. ANTHONY
ELOISE B. CRAM
PHILIP H. HARTZ
WILLIAM G. SAWITZ

Twenty-four members submitted their resignations during the year, and 22 members, delinquent in dues for 1954, 1955 and 1956, were dropped from the rolls on January 1, 1957, as per Article XI of the By-laws.

(4) *Letters to the Council.* On January 31, proposals were made concerning the place and date for the 1958 annual meeting. The Council voted unanimously to hold the meeting in Miami Beach, November 5 to 8, 1958.

On March 29 two memoranda on the financial condition of the Society were sent out. It was decided to add this item to the agenda of the annual meeting. On May 31, the Council was informed, among other items of Society business, of the resignation of Lewis W. Hackett as Editor of the JOURNAL. In August, plans for the annual meeting were detailed.

(5) *Other activities.* During the year, with the approval of the Council, a revision of the mailing list of the NEWS was begun, a return slip for continuation on the "free" list being included in the June issue. At once affirmative cards began to pour in, many with appreciative comments. However, a large number of cards have not been returned and, after an additional intimation, these recipients will be dropped. On the other hand, a large number of new requests have been received and will be added to the list.

In June, the Council approved the signing of a proposed contract with the International Cooperation Administration, whereby this organization will underwrite up to nine membership fees for those of their present and past trainees who are interested in tropical medicine and hygiene. A letter of invitation was drawn up and approved by the ICA, which will be sent to those whose names are submitted by the organization.

Other activities in connection with the various Committees, will be reported under the appropriate headings.

Respectfully submitted,
ROLLA B. HILL,
Secretary

TABLE 1

The Journal 10 years ago and today

	1947	1956
Papers published	83	134
Pages in the volume	804	1,142
Pages paid by contributors	398	242
Pages charged to the Society	406	900
Cost of printing, per page	$9.81	$16.62
Membership subscriptions	877	1,101
Nonmember subscriptions	1,215	1,267
Total revenue	$10,584	$26,287
Production costs	6,863	24,073
Surplus (divided 50–50 with the publishers)	$3,721	$2,214

The report of the Secretary was accepted as read.

The report of the Editor of the AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE was presented as follows:

Now that the JOURNAL is about to pass into new editorial hands it is an appropriate moment to review briefly some of the changes which have taken place over the last decade. The earliest statement from our publishers which I have in my files dates back just 10 years. Table 1 gives some interesting contrasts and also a few surprising resemblances.

Membership in the Society has increased some 25 per cent since 1947, and the Journal contains 40 per cent more matter, but its circulation is only 13 per cent larger than 10 years ago. Since subscriptions bring in most of the revenue, the margin of income over expenses has been narrowing all the time, and more and more precipitately. The cost of production of the Journal was only 64 per cent of the income in 1947, leaving a tidy surplus of 36 per cent to help the Society build up its comfortable reserve; the publishing cost is now 92 per cent of total receipts, up 20 per cent from last year, cutting our surplus in half. The cost of printing alone went up 26 per cent in 12 months, a good example of the general inflationary trend. The Society will probably have to adjust to thinner issues of the Journal.

Not to strike too somber a note in this farewell address, I should add that we are still in the black

TABLE 2

Financial statement

	Vol. 4–5 (6 issues from Sept. 1955–July 1956)	Vol. 5–6 (6 issues from Sept. 1956–July 1957)
Income:		
Paid subscriptions	18,580.00	19,245.82
Space sales for advertising	2,060.00	1,731.18
Paid by contributors for extra pages	2,642.12	4,356.00
Net from reprint sales	1,007.98	553.99
Back-volume sales	445.00	400.00
Total income	24,735.10	26,286.99
Production cost:		
Printing of text	15,356.12	18,981.62
Printing advertisements	667.00	526.30
Distribution and other costs	1,195.00	1,190.00
Overhead	2,900.00	3,375.00
Total cost	20,118.12	24,072.92
Surplus (income less cost)	4,616.98	2,214.07
Amount due ASTMH (50%)	2,308.49	1,107.04
Circulation and size:		
Paid subscriptions (members)	1,029	1,101
Paid subscriptions (nonmembers)	1,256	1,267
Total paid subscriptions	2,285	2,368
Pages:		
Text	1,162	1,142
Advertising	65	59
Total pages	1,227	1,201
Average cost of printing a page of text	$13.22	$16.62

by a fair though dwindling margin. We shall be in trouble only if costs continue to rise as rapidly as they have done in recent years. If our circulation has not grown spectacularly, our Journal is getting to be more widely known abroad: during the past year manuscripts came from 23 countries outside of the U. S., 17 of them tropical. We have penetrated the Iron Curtain: one of these manuscripts was submitted from Peking, and we have established an exchange with Moscow.

Table 2 presents the financial report for the year just ended, together with last year's figures for comparison. Notable is the amount received from contributors for extra pages; larger, I believe, than for any previous year. This offsets some decrease in other revenue and has provided good fat journals for all six issues, averaging 190 pages.

Table 3 presents the flow of manuscripts for the last 7 years, and Table 4 their distribution by field of interest and angle of approach. There is a remarkable consistency about these figures from year to year, unexpected with such small numbers, and little significant trend appears. Interest in helminths seems to have grown at the expense of the protozoa, and obviously the nutritionists do not consider our Journal an appropriate outlet. Epidemiology has become our outstanding interest.

Under a recent policy approved by the Council and the Editorial Board, the JOURNAL has begun to publish review papers covering broad fields of current interest to all our readers. Three have already appeared: on malaria distribution in the world today (P. F. Russell, Nov. '56); on tropical sanitation (J. M. Henderson, Jan. '57); and on amebiasis (H. E. Meleney, May '57). Three more are in preparation (on nutrition in the tropics, arbor viruses, and American trypanosomiasis), and two others are in contemplation: on yaws eradication, and on population and public health in the tropics. These articles, usually longer than the average paper, occupy extra pages of the Journal paid for out of the interest on the Society's invested funds.

In closing, I thank the contributors for giving me the best they had and for their almost invari-

TABLE 3

Flow of manuscripts over 7 years

Manuscripts	V. 31 1951	V. 1 1952	V. 2 1953	V. 3 1954	V. 4 1955	V. 5 1956	V. 6 1957
Submitted during year	143	148	177	147	164	149	118*
Declined or withdrawn	35	32	38	27	37	28	31†
Accepted	108	116	139	120	127	121	87†
On hand from last year	58	65	63	90	82	84	80†
Available for publication	166	181	202	210	209	205	167†
Published during year	101	118	112	128	125	125	134
Postponed till next year	65	63	90	82	84	80	—

* Through September 1957. In 1956, 55 manuscripts were submitted in the last quarter.
† Estimate.

TABLE 4

Subjects and approach of manuscripts over 7 years

Manuscripts published	1951	1952	1953	1954	1955	1956	1957
Subjects							
Protozoa	29	49	50	47	28	29	26
Animal hosts and vectors	13	23	23	21	33	30	21
Helminths	19	17	16	25	31	27	33
Viruses & rickettsias	14	18	7	13	10	21	22
Bacteria & fungi	14	7	9	13	16	15	11
Nutrition	4	0	0	1	3	1	0
Others	8	4	7	8	4	2	21
Totals	101	118	112	128	125	125	134
Approaches							
Biology-Taxonomy	1	9	9	4	3	5	5
Clinical	30	28	31	37	40	33	25
Epidemiology & statistics	19	28	23	38	31	39	41
Experimental, Field	7	5	5	0	0	2	3
Experimental, Laboratory	39	28	30	30	29	32	31
Prevention & control	2	14	11	12	16	9	5
Others	3	6	3	7	6	5	24
Totals	101	118	112	128	125	125	134

ably appreciative and cooperative spirit in the face of candid criticism and exasperating emendation. I thank Dr. Florence M. Frost for her patient assistance in laborious tasks connected with producing a clean printed page and papers reasonably free from solecisms. I feel an overwhelming sense of obligation and gratitude to my conscientious, able and judicious Board of Consultants. They have supported me in some tough situations and to their constructive criticism and advice is due the quality of the Journal as it now stands.

Respectfully submitted,
L. W. HACKETT
Editor

The report of the Editor was accepted as read, with appreciation and thanks for a superlatively performed operation during his tenure of the Editorship.

The report of the Editor of TROPICAL MEDICINE AND HYGIENE NEWS was presented as follows:

During the past year we have published six issues on schedule with 20 to 28 pages in each. No fundamental change was made in the format or in coverage. An attempt was made to encourage further contributions of news items by members

and friends and this was moderately successful. Also, pictures were used more frequently than in the past because it was felt that this improves the appearance of the news-letter and increases reader interest. Starting with the February issue, which followed publication of the Membership List, the names and addresses of all new members are published as well as all changes of address of members. These are printed on one side of the page only and in the same type as that used for the Membership List so that members may, if they wish, keep their Membership List up to date.

The Membership List was printed in December 1956 and distributed with the December issue of the News. The list of names and addresses was furnished to the editor by the Secretary-Treasurer and the List was composed and printed in Washington. The cost of the Membership List was about $500.00.

As in the past, publication of the News was made possible by income from advertising and by support from the American Foundation for Tropical Medicine. Burroughs-Wellcome and Co., Winthrop Laboratories, and Pfizer Laboratories continued to advertise in the News, Burroughs-Wellcome taking eight pages. To this list were added Shell Chemicals Corporation and Appleton-Century Crofts Inc.

The American Foundation for Tropical Medicine continued to distribute the News to its mailing list in return for which it defrayed a share of the cost of each issue.

"Health Hints for the Tropics" continued in great demand and brought in an estimated $350.00 in profit to the Society. A reprinting has been ordered for next January. It is planned to make minor revisions in the text at that time.

We end the year with assets equal to those on hand in 1956. But for the expense of the Membership List, assets would have been increased by about $500.00. The good financial report for 1956–1957 was due to success in securing advertising. The appointment of Dr. K. D. Quarterman to be in charge of advertising for the News was welcomed and his efforts to increase advertising in the News are greatly appreciated. It is to be hoped that the list of advertisers can be increased in 1957–1958 since it is felt that we should try to secure at least three ads per issue.

The editor wishes to acknowledge the help and cooperation of the Secretary-Treasurer and the Editor of the Journal, and he solicits the criticism and suggestions of the Council and Members.

Respectfully submitted,
LOUIS J. OLIVIER
Editor

The report was accepted as read, with a vote of appreciation and thanks to the Editor for his excellent work.

The Treasurer's report was presented as follows:

The independent auditor's report is shown below.

October 15, 1957

The Officers and Councilors
The American Society of
Tropical Medicine and Hygiene
Miami, Florida

Gentlemen:

I have examined the financial records of *The American Society of Tropical Medicine and Hygiene*, Miami, Florida, and *Tropical Medicine and Hygiene News*, Bethesda, Maryland, for the fiscal year ended September 30, 1957 and now submit as my report the exhibits, schedules and comments listed in the preceding index.

SCOPE OF EXAMINATION

My examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as I considered necessary in the circumstances to enable me to render an independent accountant's opinion on the fairness of the representations in the statements showing the assets and liabilities of the organization at September 30, 1957, and its operating results for the fiscal year then ended.

The bank balances were confirmed by direct communications with the depositories. All cash receipts and disbursements were traced through the bank accounts. All other current assets, with the exception of the inventory of "Health Hints for the Tropics" included at a cost of $154.00, were either computed by me or otherwise satisfactorily verified. The United States Savings Bonds in the amount of $20,198.00, stated at cost, were inspected at the Coconut Grove Bank and found to be in the name of the Society. There were no changes in the fixed assets during the year.

The current liability of $2,010.70 represents the expenses for the fiscal year incurred by Dr. L. W. Hackett in operating the editorial office of the Journal. The liability was verified by reviewing in detail the expenses submitted by Dr. Hackett which were found to be in order and comparable with the expenses for the prior fiscal year.

I made sufficient tests of operating accounts to satisfy myself that accepted accounting practices

has been followed and that the transactions for the year had been properly and consistently classified. As to receipt of membership dues, it was not practicable under the scope of this engagement to extend the review beyond ascertaining that the receipts as reported on the books had been deposited in the Society's bank account.

ACCOUNTANT'S OPINION

In my opinion, the accompanying balance sheets and related cash receipts and disbursement statements fairly present the assets and liabilities of *The American Society of Tropical Medicine and Hygiene*, Miami, Florida, and *Tropical Medicine Hygiene News*, Bethesda, Maryland, at September 30, 1957 resulting from cash transactions, and the income collected and the expenses disbursed for the fiscal year then ended, and are presented on a basis consistent with that of the preceding year.

W. KEITH WHEELING
Certified Public Accountant

Exhibit A

The American Society of Tropical Medicine and Hygiene
Miami, Florida

COMPARATIVE BALANCE SHEETS
September 30, 1957 and 1956

Assets

	September 30, 1957		*September 30, 1956*	
Current assets:				
Cash on hand and in bank (Exhibit B)	$3,359.64		$2,213.32	
Account receivable (Note 1)	1,107.04		1,513.32	
Interest receivable, U. S. Savings Bonds (Schedule 1)	179.40	$4,646.08	179.40	$3,906.04
Investment assets:				
U. S. Savings Bonds, at cost (Note 2) (Schedule 1)				
Series J (Maturity value $275.00)	$198.00		$198.00	
Series K (2.76%)	20,000.00		20,000.00	
	$20,198.00		$20,198.00	
Tropical Medicine and Hygiene News (Note 3) (*Exhibit C*)	1,828.73	22,026.73	1,832.51	22,030.51
Fixed assets:				
Office equipment		472.24		472.24
Total assets		$27,145.05		$26,408.79

Liabilities and Net Worth

Current liabilities:				
Editorial office expense for fiscal year due Dr. Hackett (*Exhibit B*) (Schedule 1)		$2,010.70		
Deferred credits to income:				
Prepared dues		61.50		$60.44
Total liabilities		$2,072.20		$60.44
Net worth				
Retained income (Note 4)				
Balances, September 30, 1956 and 1955	$25,876.11		$25,542.19	
Increase for fiscal years ended September 30, 1957 and 1956 (Note 3)	(1,275.50)		333.92	
Balances, September 30, 1957 and 1956	$24,600.61		$25,876.11	
Investment in office equpiment	472.24	25,072.85	472.24	26,348.35
Total liabilities and net worth		$27,145.05		$26,408.79

Note 1. The account receivable represents the estimated profit from the publication of four issues of the *American Journal of Tropical Medicine and Hygiene* by the Williams and Wilkins Company.

Note 2. Interest on U. S. Savings bonds, Series J, is available for current expenditures; interest on Series K bonds is available for reviews in the *Journal* and for publishing abstracts of papers on the program.

Note 3. The investment in *Tropical Medicine and Hygiene News* as of September 30, 1956 and the increase in retained income for the fiscal year then ended has been increased in each instance by $222.00 which represents the inventory of "Health Hints for the Tropics" on that date which was not included in the previous report. The adjustment was made on the report to give a better comparison between the last two fiscal periods.

Note 4. Of the $24,600.61 in retained income at September 30, 1957 $2,030.92 is unappropriated as of that date, the remainder having been appropriated for:

Extra pages for the *Journal*	$1,904.38
Life membership fee of Dr. Charles A. Werner invested in U. S. Savings bonds, Series J	198.00
Investment in U. S. savings bonds, Series K, the interest on which is available for reviews in the Journal and for publishing abstracts of papers on the program	20,000.00
Interest on Series K bonds, collected to September 30, 1957, less expenditures to that date (see *Exhibit A, Schedule 1*)	467.31
Total appropriated retained income	$22,569.69

Exhibit A, Schedule 1

The American Society of Tropical Medicine and Hygiene

Miami, Florida

INVESTMENTS IN U.S. SAVINGS BONDS

September 30, 1957

Serial number	*Date of purchase*	*Cost*	*Maturity value*	*Accrued interest*
Series J				
Q 21413 J	6-29-54	$18.00	$25.00	
Q 21414 J	6-29-54	18.00	25.00	
Q 21415 J	6-29-54	18.00	25.00	
C 93631 J	6-29-54	72.00	100.00	
C 93632 J	6-29-54	72.00	100.00	
Total investment Series J		$198.00	$275.00	
Series K				
M 91038 K	4-14-53	$1,000.00	$1,000.00	$13.80
M 91039 K	4-14-53	1,000.00	1,000.00	13.80
M 91040 K	4-14-53	1,000.00	1,000.00	13.80
M 91041 K	4-14-53	1,000.00	1,000.00	13.80
M 195142 K	6-29-54	1,000.00	1,000.00	9.20
V 26490 K	6-29-54	5,000.00	5,000.00	46.00
V 26514 K	7- 9-54	5,000.00	5,000.00	34.50
V 26515 K	7- 9-54	5,000.00	5,000.00	34.50
Total investment Series K		$20,000.00	$20,000.00	$179.40
Total investments in U. S. Savings Bonds		$20,198.00	$20,275.00	

Notes:

(1) There were no changes in the bonds held during the fiscal year ended September 30, 1957.

(2) All bonds are in the name of The American Society of Tropical Medicine and Hygiene and are

kept in a safety deposit box in the Coconut Grove Bank, Miami, Florida, where they were counted on October 8, 1957 in company with the Secretary-Treasurer, Dr. Hill.

(3) The interest earned on the Series J bonds is available for current expenditures. However, no interest will be received until maturity date unless the bonds are redeemed prior to that time. However, the bonds increase in redemption value each year. As of September 30, 1957, the total redemption value of the Series J bonds was $208.71.

(4) Interest on Series K bonds is available for reviews in the JOURNAL and, for publishing abstracts of papers on the program. Interest available for this purpose is summarized below:

Total interest available as of September 30, 1956	$901.33
Collections during fiscal period	552.00
	$1,453.33
Expenditures during current fiscal period	986.02
Balance of interest available as of September 30, 1957	$467.31

Exhibit B

The American Society of Tropical Medicine and Hygiene

Miami, Florida

COMPARATIVE STATEMENTS OF CASH RECEIPTS AND DISBURSEMENTS

For the fiscal years ended September 30, 1957 and 1956

	Year Ended September 30, 1957		*Year Ended September 30, 1956*	
Beginning cash balances, September 30, 1956 and 1955		$2,213.32		$2,009.66
Receipts:				
Membership dues:				
1958	$61.50			
1957	8,504.00		$60.44	
1956	278.50		8,426.00	
Prior years	40.00	8,884.00	175.78	8,662.22
The *American Journal of Tropical Medicine and Hygiene* profit for calendar years 1956 and 1955		1,937.57		2,788.45
Profits from annual meetings at New Orleans, Louisiana (1957) and Boston, Massachusetts (1956)				
Share of income from exhibits	$328.19		$150.69	
Registrations	108.47		259.60	
Refunds on advances to local committees on arrangements	300.00	736.66	50.00	460.29
Interest earned on U. S. Savings Bonds		552.00		552.00
Burroughs-Wellcome Company contribution to New Orleans meeting				300.00
Commemorative medal sales				49.50
Refund from Dr. Larsh for unused portion of expense advance New Orleans meeting		11.71		
Refund from insurance agent covering unused portion of old Secretary-Treasurer's fidelity bond due to change in officers		23.13		
Miscellaneous transactions in connection with dues and subscriptions		35.84		
Reimbursement of unused portion of petty cash advanced to Dr. Larsh upon completion of term as Secretary-Treasurer		7.69		
		$14,401.92		$14,822.12

Disbursements:				
Office expenses (Schedule 1):			$1,912.34	
Editorial (Note 1) Secretary-Treasurer	3,318.26		3,606.59	
Total office expenses (Note 1)	$3,318.26		$5,518.93	
Burroughs-Wellcome Company contribution to New Orleans meeting			300.00	
Williams and Wilkins for subscriptions to the Journal	6,738.00		6,532.00	
Publication of abstracts (*Exhibit A, Schedule 1*)	986.02	$11,042.28	257.87	12,608.80
Ending cash balances, September 30, 1957 and 1956		$3,359.64		$2,213.32
Consisting of:				
Cash in bank		$3,359.64		$2,188.86
Cash on hand (Note 2)				24.46
Total cash on hand and in bank (*Exhibit A*)		$3,359.64		$2,113.32

Note 1. The editorial expenses for the fiscal year ended September 30, 1957 had not been reimbursed to Dr. Hackett as of that date. However, the editorial expenses have been included on *Exhibit B, Schedule 1*, in order to provide comparison between the expenses of the two fiscal years.

Note 2. Dr. Hill, the present Secretary-Treasurer, keeps no petty cash fund as his needs for petty cash have been so infrequent he prefers to expend his own funds and reimburse himself periodically by check. For each reimbursement, he attaches a schedule backed up by the usual paid vouchers, for the files.

Exhibit B, Schedule 1

The American Society of Tropical Medicine and Hygiene

Miami, Florida

OFFICE EXPENSES

For the Fiscal Years Ended September 30, 1957 and 1956

Expenses	*Year ended September 30, 1957*		*Year ended September 30, 1956*	
	Editorial	*Secretary-Treasurer*	*Editorial*	*Secretary-Treasurer*
Secretarial assistance	$773.00	$1,400.00	$693.37	$2,400.00
Honorarium, assistant to editor	750.00		787.50	
Travel and meetings	319.37	180.00	243.10	316.15
Postage	133.60	224.28	121.70	286.90
Printing	25.17	766.09	32.29	214.40
Office supplies	3.35	81.77	17.60	54.48
Telephone and telegraph	6.21	3.77	16.78	2.97
Auditing fee		200.00		175.00
Medals				17.50
Freight and express		42.34		32.20
Corporate registration		30.00		30.00
Contribution to National Society for Medical Research		25.00		25.00
Insurance		156.25		51.99
Moving Secretary-Treasurer's equipment to Miami, Florida		164.14		
Miscellaneous		44.62		
Total office expenses	$2,010.70*	$3,318.26	$1,912.34	$3,606.59

* As of September 30, 1957, the editorial expenses for the fiscal period then ended had not been reimbursed to Dr. Hackett. However, to provide a comparison between the fiscal years, these expenses have been shown on this schedule.

Exhibit C

Tropical Medicine and Hygiene News

Bethesda, Maryland

COMPARATIVE BALANCE SHEETS

September 30, 1956 and 1957

Assets

	September 30, 1957	*September 30, 1956*
Current assets:		
Cash on hand and in bank (*Exhibit D*)	$1,598.41	$1,438.99
Accounts receivable	34.25	124.05
Stamps and postage deposit on metered mail	42.07	47.47
Inventory ("Health Hints for the Tropics") (Note 1)	154.00	222.00
Total assets	$1,828.73	$1,832.51
Net worth		
Retained earnings:		
Balances, September 30, 1956 and 1955	$1,832.51	$1,195.38
Increase for years ended September 30, 1957 and 1956	(3.78)	637.13
Total Net Worth (*Exhibit A*)	$1,828.73	$1,832.51

Note 1. Inventory of "Health Hints for the Tropics" at estimated cost of $222.00 was not included in the balance sheet of September 30, 1956 shown in last year's report. The balance sheet of September 30, 1956 has been adjusted to reflect this inventory and to provide a proper comparison with the balance sheet of September 30, 1957.

Exhibit D

Tropical Medicine and Hygiene News

Bethesda, Maryland

COMPARATIVE STATEMENTS OF CASH RECEIPTS AND DISBURSEMENTS

For the Fiscal Years Ended September 30, 1957 and 1956

	September 30, 1957		*September 30, 1956*	
Beginning cash balances, September 30, 1956 and 1955		$1,438.99		$946.50
Receipts:				
Advertising	$1,875.00		$1,500.00	
Sale of supplements	740.87		1,144.95	
American Foundation for Tropical Medicine	614.42	3,230.29	623.98	3,268.93
		$4,669.28		$4,215.43
Disbursements:				
Printing of *Tropical Medicine and Hygiene News* and membership list (Note 1)	$1,334.40		$978.52	
Printing of "Health Hints for the Tropics"	281.91		700.64	
Copy preparation of *News* and list (Note 1)	813.50		472.90	
Postage and express	118.94		155.42	
Office supplies (Note 1)	244.02		29.89	
Secretarial help	125.29		172.00	
Deposits on postage meter account	140.00		130.00	
Travel expenses			127.46	
Miscellaneous	12.81	3,070.87	9.61	2,776.44
Ending cash balances, September 30, 1957 and 1956		$1,598.41		$1,438.99

Consisting of:		
Cash in Bank of Bethesda	$1,595.80	$1,434.63
Cash on hand	2.61	4.36
Total Cash on Hand and in Bank (*Exhibit C*)	$1,598.41	$1,438.99

Note 1. The increased disbursements indicated in these accounts were made in connection with the preparation of a new membership list during the fiscal period.

As stated in the auditor's report above, our total assets and net worth remained substantially the same as in 1955 and 1956. Cash receipts were $400 less than in 1956, and expenses as shown were $1,500 less but, when outstanding accounts of a little over $3,000 are paid, our total expenses will be some $1,500 more than in 1956.

At the end of the fiscal year our bank balance was $3,359.64 but, subtracting the $3,000 in unpaid bills, our balance will be around $350. Against this real balance the Council has earmarked $2,371.69 (extra pages for the JOURNAL, $1,904.38; reviews and abstracts, $467.31) so that our deficit spending is over $2,000. However, at the end of the calendar year, we should receive some $1,500 as our share of the JOURNAL profits, so that our true deficit is less by that amount. It should be noted, however, that the profit from the JOURNAL is used to cover editorial expenses.

Our annual expenses, principally for the office of the Secretary-Treasurer, now run to about $4,000 in spite of the strictest economy. Of the dues paid by our members we retain approximately $2,200 for Society expenses. The expenses of the editorial office, as noted above, have hitherto been paid from the JOURNAL profits, but with the alarming drop in revenue from this source (from $2,800 in 1955 to $1,900 in 1956, to an estimated $1,500 in 1957) a part of this will have to be paid from our regular budget. Also, in 1955, the NEWS was able to turn $1,000 into the treasury, but was not able to do so in 1956, and will not do this in 1957.

We therefore need an immediate additional $1,800 in revenue and, if inflation continues, will need more. It does not seem possible to reduce our present level of expenditure and keep going as an active Society.

Unless we wish to begin spending our accumulated savings, which seems unwise, or unless other sources of revenue are discovered, which seems unlikely, an increase in our annual dues is inevitable.

Recommendations. 1. That the annual dues be increased to $10.00 beginning in 1959. 2. That the Secretary-Treasurer be authorized to spend during the next fiscal year for secretarial assistance a sum not to exceed $200 per month.

Respectfully submitted,
ROLLA B. HILL
Treasurer

The Treasurer's report was accepted as read, with a vote of thanks to the Treasurer.

In discussion of the report, it was brought out that our investments in U. S. Government bonds yield 2.76% per annum, while other "safe" investments yield up to 4.5%. In view of the urgent need for additional funds for carrying on the activities of the Society, the incoming president was authorized to appoint a committee of two persons to assist and advise the treasurer in reorienting and reorganizing our investment portfolio, this action to be taken as soon as possible.

The two recommendations in the report were approved.

REPORTS OF COMMITTEES

Bailey K. Ashford Award. The Committee, following agreement with the sponsors, The Eli Lilly Company, to raise temporarily the age limit to 38 years, considered the names of various persons nominated by members of the Society, and voted to present this award to Paul P. Weinstein.

Development and Needs. The Chairman reported that the National Research Council is still awaiting a decision from a private foundation on its application for funds. Nevertheless, it seems possible that the project will be activated in the not-too-distant future, and the Committee was authorized to present a list of names to the Division of Medical Sciences for consideration of one as Director of the new enterprise when it becomes a reality.

Honorary Membership. The Committee has no nomination to suggest to the Council this year. This conservative action is due to our feeling that such membership should be a high privilege not to be lightly bestowed.

Membership. The Committee examined the credentials and approved 64 applicants for membership in the Society. The total membership is 1,229:

14 honorary members, 7 emeritus members, 1 life member, 1076 active members, and an additional 131 members delinquent in dues for 1 or more years. Continuing the policy of recent years, no attempt has been made by the Committee to solicit new members.

Program. Early in the year, the Committee agreed to follow the usual general plan for the annual meeting, including a symposium, which was arranged for by the president. The announcement of the meeting and call for papers were sent out on April 10. The prompt response of contributors is much appreciated. Seventy three requests to present papers, five to show motion pictures and five to mount scientific exhibits, were accepted. It was necessary to schedule two double sessions in order to accomodate all participants.

Public Relations. The activities of the Public Relations Committee have been carried on through correspondence. It has not seemed appropriate to call the membership together as a group. Early in the year the incoming president was queried as to his suggestions as to activities. He indicated his concern with establishing a broader concept of tropical diseases to encompass more of the infectious conditions of the tropics but felt that our Committee could accomplish its best purpose by communicating with the Appropriation Subcommittees of the House and Senate in support of the national need for research in the general field of infectious disease, more especially as related to the future activities of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, the Communicable Disease Center, and the Gorgas Memorial Laboratory. Since monies for training and research in the general field of interest of our society are provided by the Federal Government through the mechanism of the grants-in-aid program of the NIH, it was felt the Committee could most effectively concern itself with furnishing information to the chairman of the Senate and House Committees having to do with appropriations in support of medical research. Accordingly, letters were forwarded to the Honorable Lister Hill, chairman, the Senate Committee on Labor and Welfare, and to the Honorable John E. Fogarty, chairman, Labor and Health, Education and Welfare Subcommittee, House of Representatives. It was pointed out that infectious diseases in general continue to cause unacceptable tolls in morbidity, disability and death in our country, and abroad in the military forces, missions and the civilian populations in which we have a profound interest. It was further pointed out that, while we have accomplished the most in the prevention, control and treatment of bacterial diseases there are, nevertheless, unanswered questions especially as concerns such diseases as the streptococcal infections. Emphasis was placed upon the developing knowledge of the viral diseases and the urgent need to continue and expand research, not only in the viral disease field but also in the general field of the parasitic diseases.

One member of our Committee, Dr. Harold W. Brown, also served as Chairman, Public Relations Committee, of the American Society of Parasitologists. Thus, liaison was maintained between the two Committees and there was a full exchange of correspondence to Senator Hill and Representative Fogarty in support of legislation and appropriations to provide funds for research and training. Additionally, there was an exchange of information and letters with Dr. Walsh McDermott, who has been very active in support of Senate and House action for adequate appropriations for the Institute of Allergy and Infectious Diseases.

Dr. McDermott and Dr. René Dubos were finally permitted to testify before the Appropriations Subcommittee in support of appropriations for the NIAID on May 6, 1957. Prior to this it had developed that Congressman Fogarty had supported the appropriations bill and was able to put it through both the SubCommittee and the Committee of the House, but that the bill faced a "battle on the floor." A letter was addressed to Mr. Fogarty on April 3, 1957, calling his attention to the earlier letter expressing the views of the Society and thanking him for the vigorous position he had taken in support of the legislation.

Subsequently, the appropriation bill did pass the House and received approval of the Senate Appropriations Committee and eventually full Senate approval. As a result, approximately four million dollars was appropriated over and above the budget for the previous year.

It is felt that your Committee may have had some influence in this favorable action of the Congress to provide additional support for research and training in allergy and the infectious diseases. It must be observed, however, that the close liaison and joint activities with the Public Relations Committee of the American Society of Parasitologists and with Dr. Walsh McDermott gave much strength to these efforts.

All of the above reports were accepted, with appreciation for the efforts of the various committees.

The Council approved a Resolution directing the Program Committee to decline papers from members in arrears of dues, and also directed the

Committee to limit the number of papers accepted to that which can be presented at single sessions.

REPORTS OF REPRESENTATIVES TO ORGANIZATIONS

National Research Council. Submitted by Leon Jacobs.

I attended the annual meeting of the Division of Medical Sciences, National Research Council, on May 17, 1957, at which time I submitted a written report of the activities of the Society, stressing the value of the JOURNAL of the Society and the importance of this Society itself in serving as the organization stimulating research in tropical medicine in the United States.

Your attention is called to page 17, sub-section C of the agenda of the meeting, concerning the establishment of an organization for a survey of the field of tropical medicine in the United States. I specifically asked about this point at the meeting in the hope that I would have definite information to relay to you concerning the availability of funds for this purpose. I was told that while funds were expected, no definite commitment had yet been obtained.

The report was accepted as read.

OTHER OLD BUSINESS

Election of Officers and Councilors for 1957–58. The Nominating Committee submitted a slate which was mailed with a ballot to all voting members of the Society. The votes received were tabulated by the tellers, Drs. D. L. Augustine and T. H. Weller, with the following result:

President elect	L. W. HACKETT
Vice-President	P. C. BEAVER
Councilors	P. F. RUSSELL
	J. E. SMADEL

NEW BUSINESS

Committee appointments. The President appointed J. A. Scott and J. C. Swartzwelder to serve as the Resolutions Committee.

The Council elected the following members to serve on the Nominating Committee for 1957–58: L. B. Hall, J. C. Swartzwelder, and T. H. Weller.

The Council elected J. M. Andrews to serve on the Committee on Award of the Walter Reed Medal for a term of 6 years.

Contribution to the National Society for Medical Research. The Council voted to contribute again $25.00 to this Society.

Ad hoc Committees for 1957–58. The Council authorized the President to appoint such committees as he deemed necessary.

Election of Emeritus Members. The Council approved the requests of, and elected to Emeritus Membership, Randolph Lyons and Eugene R. Kellersberger. By mail ballot earlier in the year, S. Bayne-Jones and Alfred Gage were also elected.

Official Representation to Congresses. In response to an invitation to send an official representative to the Ninth Pacific Science Congress, which convenes in Bangkok in November, 1957, Wm. McD. Hammon was chosen to represent the Society.

Following an invitation from the Congresses of Tropical Diseases and Malaria to be represented, when it meets in Lisbon in September 1958, the Council elected J. M. Andrews as an official representative.

Report on Contract with the International Cooperation Administration. The President, previously authorized by the Council, announced that he had signed a contract with ICA, whereby the ICA will sponsor up to nine members for the Society, from their present or past trainees, for a maximum period of 3 years.

Election of an Editor for the JOURNAL. The Council received, and with deep regret accepted, the resignation of L. W. Hackett as its Editor. After discussion, Martin Frobisher was unanimously elected Editor, to take office at a time agreed upon between him and Dr. Hackett.

Report on the Louisiana State University Fellowship Program in Tropical Medicine and Parasitology. By invitation, Dr. Meleney gave a short report on the accomplishments of this program in the 3 years of its operation. As a result of its successful operation, support has been received to continue the program through 1962.

Election of Fellows. The Council elected the following members to fellowship:

CARLOS A. ALVARADO
PAULO C. A. ANTUNES
LOUIS J. OLIVIER
RICHARD M. TAYLOR

Change in format of the JOURNAL. To help offset the increasing cost of publication of the JOURNAL, the Council approved the proposal of the Editor to change the format from a one-column to a two-column page.

There being no further business, the meeting adjourned.

MINUTES OF THE ANNUAL BUSINESS MEETING

The annual business meeting of the Society was held November 2, in the ballroom of the Benjamin Franklin Hotel, with President J. M. Andrews presiding.

The minutes of the fifth annual meeting in New Orleans, November 3, 1956, were approved as published in the JOURNAL.

The reports of the Secretary-Treasurer, the Editor of the JOURNAL, and the Editor of the NEWS were approved as read.

Report of Council Actions. The results of the election, by mail ballot, of Officers and Councilors were announced by the Secretary.

The Secretary announced the following council actions:

1. Approval of an increase in dues from $8.00 to $10.00 annually, beginning in 1959.
2. Appointment of a Nominating Committee.
3. Approval of a contribution of $25.00 to the National Society for Medical Research.
4. Appointment of Wm. McD. Hammon as official representative to the Ninth Pacific Science Congress, and of J. M. Andrews as an official representative at the International Congresses of Tropical Diseases and Malaria.
5. Election of Martin Frobisher as Editor of the *American Journal of Tropical Medicine and Hygiene.*
6. Election of four active members as Fellows.
7. Signing of a contract with the International Cooperation Administration.
8. Selection of Miami Beach for the next annual meeting, Nov. 5–8, 1958.

Report of the Resolutions Committee (J. Allen Scott, Chairman.)

I. It is requested that the Secretary of the Society be instructed to write and send resolutions of sympathy to the families of deceased Fellows and members of the American Society of Tropical Medicine and Hygiene. During the current year, the Society has lost Frederick G. Novy, Henrique da Rocha-Lima, Jerome Rodhain, and Kioyoshi Shiga, honorary members; Eloise B. Cram, Fellow; Sarkis B. Anthony, Philip H. Hartz and William G. Sawitz, active members.

II. To Dr. W. H. Wright, Moderator, and members of his panel, the thanks and appreciation of the Society are extended for the outstanding and enlightening symposium on *Zoonoses in the Tropics.*

III. To Dr. Louis L. Williams, Jr. we extend this Society's sincere thanks and highest esteem for his able presentation of the 22nd Annual Charles Franklin Craig Lecture, *"Malaria Eradication—Growth of the Concept and its Application."*

IV. To the organization and individuals who have served as hosts for the Sixth Annual Meeting of the Society held in Philadelphia, Pa., October 30–November 2, 1957, or otherwise furnished helpful assistance:

A. The officers and members of the Society are deeply appreciative of the assistance and hospitality extended by the Jefferson Medical College of Philadelphia, the School of Medicine of Temple University, and the Schools of Medicine and of Veterinary Medicine of the University of Pennsylvania and by the members of their Faculties and staffs. THEREFORE BE IT RESOLVED that the thanks of the Society be expressed to the Deans of these institutions, Drs. George Bennett, William Parkinson, John Mitchell and Mark Allen, and through them to their respective institutions, faculties and staffs.

B. The Visitors and Convention Bureau of Philadelphia has provided secretarial help and other assistance and has otherwise contributed greatly to the success of the Meeting. THEREFORE BE IT RESOLVED that the thanks of the Society be extended to the bureau through Mr. James Morrison, Manager.

C. The Society appreciates the provision of a subvention by Merck Sharpe and Dohme for the program of Ladies' Entertainment. THEREFORE BE IT RESOLVED that the thanks of the Society be expressed to this company for its assistance.

D. The assistance and cooperation of the members of the staff of the Benjamin Franklin Hotel contributed to the success of the meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Mr. Allan F. Schnell, Sales Manager, and through him to the members of the hotel staff.

E. The interest and planning of the Committee for Ladies' Entertainment contributed greatly to the pleasure of the ladies attending the meeting. THEREFORE BE IT RESOLVED that the Society express its thanks and appreciation to Mrs. George L. Graham, Chairman, and through her to the following committee members: Mesdames Edwin S. Gault, John J. Hanlon, John H. Hodges, Frank F. Katz, Harry M. Martin, Hobart A. Reinmann, William A. Sodeman, D. H. Wenrich, Thomas F. Whayne, Chris J. Zarafonetis.

F. The foresight and planning of the Local Committee on Arrangements were essential contributions to the success of the meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Drs. George L. Graham and C. Brooke Worth, Co-chairmen,

and committee members as follows: Drs. Edwin S. Gault, John J. Hanlon, John H. Hodges, Frank F. Katz, Harry M. Martin, Andrew J. Warren, Fred B. Rogers, David H. Wenrich, Tom F. Whayne, and Chris J. Zarafonetis.

G. The Hospitality Sessions were a most important contribution to the pleasure of the individual members and guests and to the success of the meeting as a whole. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be extended to Burroughs Wellcome and Co., The Schering Corporation, and Wyeth Laboratories for providing these sessions.

H. The Commercial Exhibits added materially to the interest of the Meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Mrs. Herma H. Hoefler of the American Foundation for Tropical Medicine, who served as Chairwoman of Commercial Exhibits Arrangements, and through her to the commercial exhibitors.

I. The Scientific Exhibits were of outstanding interest. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed through the Pan American Sanitary Bureau, U. S. Army Environmental Health Laboratory, and The Armed Forces Institute of Pathology to the individuals who so painstakingly developed these exhibits.

V. Editors of Society Publications.

A. Dr. Lewis W. Hackett, Editor of the American Journal of Tropical Medicine and Hygiene, is to be commended for his efforts which have resulted in a journal of outstanding quality. It is greatly regretted that Dr. Hackett has found it necessary to resign from his position as Editor. THEREFORE BE IT RESOLVED that an expression of thanks on behalf of the Society be extended to him for his conscientious and successful service during his term of office.

B. Dr. Louis J. Olivier has ably edited the *Tropical Medicine and Hygiene News*. THEREFORE BE IT RESOLVED that an expression of appreciation of the Society be extended to him.

VI. WHEREAS the Royal Society of Tropical Medicine and Hygiene will celebrate its fiftieth anniversary at a Jubilee Banquet in London on November 12th, 1957, BE IT RESOLVED that the Secretary of the American Society of Tropical Medicine and Hygiene be requested to extend to Brigadier J. S. K. Boyd, President of the Royal Society of Tropical Medicine and Hygiene, and through him to that Society, the greetings and felicitations of this Society on this happy occasion.

Following the announcement of these various resolutions, they were approved by voice vote.

There being no further business, the retiring President relinquished the Chair to the incoming President, D. L. Augustine.

On motion, the meeting was adjourned.

Respectfully submitted for publication,

ROLLA B. HILL
Secretary-Treasurer.

SCIENTIFIC AND SPECIAL SESSIONS

Eight half-day sessions were held, with six regular sessions for the presentation of papers, a special session for the showing of motion pictures, and a special joint session with the American Society of Parasitologists for a symposium. In addition to these eight sessions, there was a dinner session, at which Norman Topping served as Toastmaster. At the dinner the Chairman of the Joseph A. LePrince Award Committee, J. M. Andrews, presented this award to L. L. Williams, Jr., and the Chairman of the Bailey K. Ashford Award Committee, L. H. Schmidt, presented this award to Paul P. Weinstein. The President, J. M. Andrews, presented an address on "Tropical Allergens and Allergic Diseases."

1:30 p.m., October 30

*1. THE TWENTY-SECOND ANNUAL CHARLES FRANKLIN CRAIG LECTURE: *Malaria Eradication—Growth of the Concept and its Application*. LOUIS L. WILLIAMS, JR., U.S.P.H.S., Retired. Introduced by JUSTIN M. ANDREWS.

2. *A Note on Malaria Eradication in Ceylon*. JOHN M. HENDERSON, Savannah, Ga.

The Ceylon Malaria Eradication Program is generally described in terms of malaria reduction, subsequent malaria resurgence and revised operational plans to achieve eradication. The paper is based on information obtained during the author's visit to Ceylon in April, 1957, for the purpose of evaluating the malaria eradication program.

†3. *Pyrimethamine and Chloroquine in Salt as a Suppressive against Sporozoite-induced Chesson-strain* vivax *Malaria*. G. ROBERT COATNEY, OLAF MICKELSON, MARTIN D. YOUNG, ROBERT W. BURGESS AND CARL I. PIRKLE, National Institutes of Health, Bethesda, Md.

* To be published in the JOURNAL.

† Abstract not published at request of author(s).

†4. *The Development of Pyrimethamine Resistance in Human Malaria.* MARTIN D. YOUNG, National Institutes of Health, Columbia, S. C.

†5. *The Effect of Pyrimethamine Resistance upon the Sporogonous Cycle of Malaria.* ROBERT W. BURGESS AND MARTIN D. YOUNG, National Institutes of Health, Columbia, S. C.

‡6. *Toxicity of Pyrimethamine (Daraprim) in Man.* RALPH JONES, JR., ULFAR JONSSON, HOWARD LESSNER AND JOHN W. FROST, University of Miami, Miami, Fla., and University of Pennsylvania, Philadelphia, Pa.

Pyrimethamine has been administered to 24 patients with polycythemia and other diseases in doses ranging from 25 mg to 300 mg per day. One patient who received 300 mg per day developed convulsions on the sixth day. A daily dose of 200 mg produced marked anorexia and severe gastrointestinal discomfort as the limiting toxic action.

Gastrointestinal complaints were the principal limiting toxicity reaction in patients taking 100 mg a day. With doses below 100 mg a day the predominating toxic effect has been bone-marrow depression. The effect on the bone marrow has been predominately in the erythroid elements. The granulocytes and platelets may also be affected. The effect on the bone marrow developed slowly, requiring several weeks to several months of continuous administration. Prolonged administration of a dose of 25 to 50 mg a day may produce alopecia, atrophy of the mucous membrane, sore, red tongue and ulceration of the mouth. All of these effects are promptly corrected by the administration of small doses of folic acid by mouth. The significance of these observations in relation to the use of pyrimethamine in the treatment of malaria are discussed.

7. *Quantitative and Histochemical Observations on Liver Lipids in Rats Infected with* Plasmodium berghei. THEODOR VON BRAND AND TERESA I. MERCADO, National Institutes of Health, Bethesda, Md.

Previous work had shown that in *Plasmodium berghei*-infected rats the glycogen functions of the liver were impaired. Because glycogen and lipids frequently are deposited in an opposite manner, a study of the latter was undertaken. Quantitative determinations showed that livers from infected animals contained, on an average, more lipids than livers from uninfected controls, regardless of whether starved animals, animals injected with Meticortelone, rats fed fructose, or rats given fructuse and Meticortelone were used. On the other hand, a positive correlation existed between increased lipid content and degree of parasitemia. The various fractions investigated (acetone-insoluble lipids, unsaponifiable material, fatty acids isolated from acetone-soluble fraction) were all more or less increased. The fatty acids from acetone-soluble lipids, i.e., essentially from triglycerides, increased most while the acetone-insoluble fraction, i.e., essentially phospholipids, increased only slightly. Histochemically, a very large increase of morphologically demonstrable lipids occurred in many infected livers giving a pattern of centrilobular infiltration. This is the region where, previously, glycogen depletion and lack of glycogen synthesis had been demonstrated. The cells in the vicinity of the portal vessels, where the glycogen relationships are essentially normal, showed less demonstrable lipid than is found in corresponding areas of control animals.

*8. *On the Structure of the Nucleus during Certain Stages in the Sporogonous Cycle in Certain Species of* Plasmodium. GORDON B. WOLCOTT, National Institutes of Health, Columbia, S. C.

9. *Nuclear Division of* Iodamoeba bütschlii *(von Prowazek, 1912) Dobell, 1919.* CHIA-TUNG PAN, Harvard University, Boston, Mass.

Of the six species of amebae living in the digestive tract of man, the process of nuclear division has been described for all except *Iodamoeba bütschlii*. In a series of stool examinations, large numbers of trophozoites and cysts of *I. bütschlii* were found in two mushy specimens from a woman who had previously lived in India. Several hundred stained preparations were made, and the process of nuclear division was studied.

The perikaryosomal granules in the interphase nucleus appear to be retracted into the karyosome at the beginning of the prophase; at the same time the karyosome becomes less dense and shows grouping of the chromosomal granules. At this time, the nucleus elongates to form the spindle and the chromosomal granules move to the metaphase plate. The two sets of chromosomal granules are gradually pulled toward opposite poles and, soon after, they coalesce into large, amorphous masses (anaphase). The paradesmose in the spindle frequently extends beyond both poles, and a granule suggestive of a centriole may be seen at each end. Near the close of the anaphase the spindle starts to constrict at its middle. The two daughter nuclei pull away from each other trailing the paradesmose behind them. The masses of chromosomal granules become denser as they approach the poles (telophase), and the perikaryosomal granules begin to reappear at the same

‡ By title.

* Abstract not published at request of author.

time. The nuclear membrane remains visible during the process of nuclear division.

10. *International Collaboration in the Integration of National Health Services.* GUSTAVO MOLINA AND P. F. DE CAIRES, Pan American Sanitary Bureau, Regional Office of the World Health Organization, Washington, D. C.

To be successful, international collaboration and assistance in the organization, development and integration of national health services must be based on a national program and plan, with services created to meet established needs in the order of their priority. The scope and speed of development of these services will depend on available resources.

International assistance has two basic elements, advisory services and fellowships for the training of key national personnel abroad. The role of the international adviser is essentially to stimulate, advise, teach, and leave a going concern when he departs.

Indices for the evaluation of international assistance must necessarily be related to the national services. While it is difficult to set up universal criteria to be applied in specific order, the presence or absence of certain fundamental requirements can be used to assess the success or failure of the help provided by the international health agency.

Rather than the development of special services for selected groups of the population, the policy of PASB/WHO is to promote the integration of a more complete health service, under a single direction, for the community as a whole.

11. *The Dawn of Medical Education in Tropical Africa.* HENRY R. O'BRIEN, Camp Hill, Pa.

In the Tropical Zone in Africa live more than 140,000,000 people. They have tremendous problems and needs in the field of health. In the past century Western science has made progress, of which we are proud, in research on Africa's diseases, and has won solid therapeutic victories. But Africa's health problems must finally be solved by Africans. Above all Africa's sick must be treated by African doctors, many and competent.

A start has been made in training numbers of aids (medical, nursing, sanitary) and in sending outstanding students to Western medical schools. Now modern medical schools are appearing in Africa itself. They are at Khartoum (Sudan), Dakar (French West Africa), Ibadan (Nigeria), Kampala (Uganda), Leopoldville and Elizabethville in the Congo, Tananarive (Madagascar), with Durban out of the tropics to the south.

These pilot runs show that physicians can be trained successfully in Tropical Africa. Organization and growth in all these countries follow certain patterns. The numbers, of schools and students alike, are pitifully small. Like Portia's candle, the medical schools in Tropical Africa cast their light a long way. But the darkness is great. Much more must be done, and quickly. We in the West have a large responsibility, and opportunity.

12. *Child Health in the Caribbean.* D. B. JOLLIFFE, Tulane University, New Orleans, La.

The problems affecting child health in various West Indian territories are outlined, based on a personal survey and on available statistics and medical literature. The socio-economic, cultural and medical etiology of the most important problems, including malnutrition (especially kwashiorkor and nutritional marasmus), infantile diarrhea and the intestinal parasitoses, are discussed. Current and future trends in preventive pediatrics are considered.

9:00 a.m., October 31

13. *On the Prepatent Periods of the Geographical Strains of* Schistosoma japonicum. H. F. HSÜ AND S. Y. LI HSÜ, State Univ., of Iowa, Iowa City.

It is of interest to know whether the geographical strains of *S. japonicum* can be differentiated by their prepatent periods in the final hosts. Four strains, the Chinese, Formosan, Japanese and Philippine were used for the study and the albino mouse was used as the experimental animal.

No significant differences were found in the prepatent periods either between the Chinese and Formosan strains, or between the Japanese and Philippine strains. However, the prepatent periods of the Chinese and Formosan strains were significantly longer than the latter two.

The results indicate that the anthropophilic Chinese, Japanese and Philippine strains are not always more closely related with one another than the Formosan, the zoophilic strain. All four may have originated from one ancestral stem, and each developed its own characteristics due to long geographical isolation.

14. Schistosomiasis mansoni *in Mice with Nutritional Fatty Liver Disease.* WILLIAM B. DEWITT, National Institutes of Health, Bethesda, Md.

Fatty-liver disease was produced by feeding young, adult, N.I.H.-strain, male mice a ration

deficient in choline and methyl-group precursors. Control animals were kept on a "complete" semi-synthetic casein diet. After the mice were on the diet for 8 weeks they were each exposed to 75 cercariae of *S. mansoni*. The infections were allowed to run an additional 8 weeks.

At autopsy the mice on the fatty-liver diet harbored about 30% fewer worms than those on the control diet. No change in sex ratio was noted. The worms from the deficient animals were poorly developed and apparently had produced only a few eggs. The growth of the animals was retarded by both the deficient diet and the schistosome infection. By the end of the experiment the infected mice on the deficient diet had gained 3.1 gm while uninfected mice on the same diet showed a gain of 4.6 gm. Comparable animals on the control diet had gained 11.9 and 13.7 gm, respectively.

Histologically, the livers from mice fed the choline-deficient diet showed massive accumulation of stainable fat within parenchymal cells, as well as numerous extracellular fatty cysts. Signs of early fibrosis were noted. Very few or, in some cases, no eggs could be found in the liver sections from infected mice on the deficient ration, while numerous pseudotubercles (70 to 200 per section) could be counted in sections from infected mice on the control diet. There was little or no schistosomal pigment present in the Kupffer's cells of mice on the fatty-liver diet which was in sharp contrast with infected mice on the complete diet.

15. *The Histopathology of Experimental Infections in Mice with* Schistosomatium douthitti. Irving G. Kagan and David R. Meranze, University of Pennsylvania and Albert Einstein Medical Center, Philadelphia, Pa.

Tissues from 108 mice experimentally infected with cercariae of *S. douthitti* for periods of 15 to 840 days were studied.

Lesions of the liver were predominately associated with the deposition of eggs. In the absence of eggs (male infection) accumulation of mononuclear cells, localized about veins, was noted. Mice cured of infection showed a return to normal in approximately 270 days.

Lesions of the spleen were also associated with deposition of eggs. A marked difference in inflammatory response toward eggs in the spleens of three host species was noted. In the absence of eggs marked congestion was noted. After cure the return to normal was not as rapid as in the liver.

In early infection, the muscularis mucosa of the intestine confined the infection to the submucosa. Although observations on how eggs escaped the intestine were made, no ulcerations of the villi could be detected.

In the lungs typical pseudotubercle lesions around eggs and adult worms were seen. In male infection accumulation of mononuclear cells around veins was the principal inflammatory response. In the infected rat a marked tissue response in the lung was noted.

Study of the bone marrow, kidney and lymph nodes in the infected animal revealed no marked change from the normal. No striking or marked change in the immune host, challenged with cercariae, could be discerned.

16. *The Development of Esophageal Varices in Mice Infected with* Schistosoma mansoni. Kenneth S. Warren and William B. DeWitt, National Institutes of Health, Bethesda, Md.

It was found that approximately 60% of young, adult, Swiss, albino, female mice developed esophageal varices within 12 weeks following exposure to 100 cercariae of *S. mansoni*. This was accompanied by hepatomegaly, splenomegaly, and portal hypertension. The varices were seen on the serosal surface of the esophagus at autopsy, and were further confirmed by latex injection of the mesenteric veins and x-ray portagrams.

Twelve weeks after infection the liver weight averaged 2.9 gm as compared to 1.9 gm in normal control mice, and the spleen size was 30 x 6 mm as compared to 19 x 5.3 mm. The portal pressure was twice that of control mice at 12 weeks, and the peritoneal cavity contained more fluid than normal. Egg counts revealed an average of 2,160 eggs per gm of liver tissue at 12 weeks, with 2,765 eggs in those with large varices and 1,550 in those with moderate to no varices. The mice harbored an average of 32 worms; 11 pair plus 10 male worms. Study showed eggs in the portal areas surrounded by fibrosis and inflammatory reaction, but no signs of generalized fatty changes.

17. *Schistosomiasis as Related to Hepatosplenomegaly in Puerto Rico.* Elmer F. Chaffee and Hector F. Rodriguez, Tropical Research Medical Laboratory, U. S. Army, San Juan, and Bayamon District Hospital, Bayamon, Puerto Rico.

Opinions vary as to the part which schistosomiasis plays in hepatosplenomegaly. Some investigators believe that malnutrition is probably the dominant factor in organ enlargement. The purpose of this investigation was to compare the incidence of schistosomiasis in patients with hepatosplenomegaly to that in patients without organ enlargement but from the same population.

Patients in the test group were selected as admitted, solely on the basis of hepatomegaly and/or splenomegaly; those in the control group were from the same wards, the only criteria for selection being the absence of organ enlargement and that they be of the same sex, number and approximate age as the test patients. Twenty-three (59%) of

the 39 patients with hepatosplenomegaly had evidence of schistosomiasis, while only 9 (23%) of the patients without organ enlargement showed evidence of infection with *S. mansoni*. The difference is statistically significant.

Thirteen of the 23 test patients with schistosomiasis had schistosomal cirrhosis, the diagnosis being established pathologically in six. In the other seven cases there was moderate to marked liver-function impairment. *S. mansoni* eggs in the stool and/or rectal biopsy, and etiologic factors such as alcoholism, malnutrition, ingestion of hepatotoxic agents, biliary system disease, and previous hepatitis were ruled out.

Since the nutritional status of these 78 patients was comparable, it seems logical to conclude that schistosomiasis is a major predisposing factor in hepatosplenomegaly in Puerto Rico.

18. *The Fecal Egg Output of* S. mansoni *in Mice.* José F. Maldonado, University of Puerto Rico, San Juan, P.R.

Mice, each exposed to 50 *S. mansoni* cercariae, and that had just started to show eggs in feces, were isolated in individual cages and all the droppings carefully collected in Stoll flasks containing the required 56 ml of 0.1 NaOH. To prevent putrefaction, 5% Formalin was added to this. The animals were kept for periods of up to 55 days, during which no less than eight egg counts were performed by modifying slightly the Stoll technique.

The number of eggs per ml of feces per female worm has been found to be 41.9 ± 16.1. An average of 3.3 fully mature females per animal were counted. The proportion of males up to now has been 4:1. Since these are not likely to contribute in any major degree to the pathology of the disease, only the female should be considered in determinations of this nature.

The work is in progress, with a goal of at least 200 mice to be studied.

19. *Studies of* Schistosomiasis mansoni *in Primates. (2) Observations on Acquired Resistance.* David H. Naimark, A. S. Benenson, José Oliver-Gonzalez and Lawrence S. Ritchie, U. S. Army Tropical Research Medical Laboratory and University of Puerto Rico, San Juan, Puerto Rico.

Three monkeys were each exposed cutaneously to 1,000 mixed cercariae of *S. mansoni*. Maximum counts of eggs in the stool occurred between 100 and 150 days, and fairly rapid decline followed. Reexposures to 500 cercariae on three occasions at 15-week intervals between days 650 and 900 did not cause any increase in egg counts. Two monkeys became completely free of eggs at 550 and 950 days. No change in egg recovery was noted after a challenge with 5,000 cercariae at 970 days. Following a second challenge with 5,000 cercariae, two animals were sacrificed at 6 days and no worms were found, while the third animal, after 23 days, harbored 27 adult and 421 immature worms.

A second group of monkeys received a series of 26 light exposures (25 to 50 cercariae) at intervals of about 1 month. Egg counts increased gradually for about 400 days in two monkeys and then declined sharply thereafter, in spite of continued exposures for another year. A third animal died of schistosomiasis, following the eleventh exposure, with a recovery of 178 worms.

The third group combined one or two exposures to 500 cercariae, with a series of 20 light ones. Resulting egg counts suggested the existence of varied levels of natural resistance. Challenges with 5,000 cercariae did not cause renewal or increase in egg recovery during a period of 85 to 100 days. Then, following a second challenge of 5,000 cercariae, animals were sacrificed at intervals of 6, 15, 23 and 43 days. Adult worms were few or absent whereas considerable numbers of immature worms and schistosomulae were encountered, though less than 10% of the cercariae used lived. Their ultimate fate is conjectural, but it would appear that resistance was being developed.

20. *Physicochemical and Serologic Studies of* Schistosoma mansoni *Antigens.* H. Kenneth Sleeman, James C. Burke, and John F. Kent, Walter Reed Army Institute of Research, Washington, D. C.

Extraction of desiccated, adult *Schistosoma mansoni* with anhydrous ether removes components that cross react with syphilitic sera without adversely affecting reactivity with homologous antiserum. The specific complement-fixing fraction appears to be conjugated protein. It is precipitated by half saturation with $(NH_4)_2SO_4$ or by dialysis against water, and is insoluble in 0.25 M trichloracetic acid under conditions described by Bolvin; it is resistant to pepsin and trypsin. Fractionation by a modification of Cohn's method 10 yields two antigens, one in fraction III-0, the other in a fraction designated I + III-3; both react in tests for lipid but not in tests for carbohydrate. The former, a nondialyzable component resistant to desiccation, contains 30% of the original N and 50% of the P. The latter, a component labile to desiccation, retains 2% of the original N and 10% of the P. Both fractions exhibit specific reactivity with homologous antiserum, and have been standardized for use with a sensitive complement-fixation procedure. Results obtained in a serologic study of patients with homologous and heterologous infections are presented, and the findings are discussed with respect to their contribution to the serodiagnosis of schistosomiasis.

21. *Treatment of* Schistosoma mansoni *Infection in Children with Leucanthone Hydrochloride (Nilodin).* H. B. Shookhoff, A. Einhorn, K. G. Dwork, and A. Fritsch, N. Y. C., Health Department, Albert Einstein College of Medicine and Bronx Municipal Hospital Center, New York, N. Y.

Lucanthone was administered to 33 children from Puerto Rico who were found infected with *S. mansoni*. Their ages were from 7 to 14 years. The dose was 20 mg per kg per day for 7 days. In 15 instances this was well tolerated. In 18 cases, it had to be reduced after a few days because of vomiting or abdominal pain. In 17 of the 18, the total dose was 1.0 gm per kg in the 7 days; in the 18th case it was 0.5 gm per kg.

Aside from the gastro-intestinal symptoms, there was only one possibly untoward effect; namely, a convulsive seizure which occurred after 4 days of the full dose. Treatment was stopped for 2 days and then resumed at 10 mg per kg without incident. Blood counts, liver function tests and urine examinations were done in all cases before and after treatment and showed no adverse effects.

Seventeen patients were followed for 3 to 6 months with an average of 3.5 stool examinations. Eggs were found in the stools of only 3 of the 17 and, in these 3, the number of eggs was greatly reduced. The remaining 16 all had negative stools; 7 were followed for 2 months with 2 stool examinations each and 9 for 1 month with a single stool examination.

Lucanthone is better tolerated by children than by adults. The results to date suggest that it is an effective therapy for children with *S. mansoni* infection.

22. *Life History of* Australorbis glabratus *as Observed under Laboratory Conditions.* Alfred Hernandez and Lawrence S. Ritchie, U. S. Army Tropical Research Medical Laboratory, San Juan, Puerto Rico.

A study of the life history of the Puerto Rican *Australorbis glabratus* is in progress, with specific aims to observe all aspects of the life history under laboratory conditions, including growth rate from hatching, age at onset of egg laying, incubation period and egg fertility, reproductive capacity by age interval and determination of life span. Attempts will be made to obtain parallel observations under field conditions. At 20, 40, 80 and 160 days of age, mean sizes for snails reared in isolation approximated diameters of 5, 10, 13 and 15 mm, respectively. Survival ratse were about 90% at 80 days and 85% at 160 days. The onset of oviposition occurred as early as 30 to 32 days from hatching and the mean ages for 10 sibling groups (total of 168 snails) ranged from 36 to 43 days, the overall mean being 39.5 days. Egg clutches averaged about 42 eggs and the extent of laying was approximately 25 eggs per snail per day.

23. *Effects of Crowding on* Australorbis glabratus *(Say) as Observed under Laboratory Conditions.* Elsie Brenes, Myron G. Radke and Lawrence S. Ritchie, U. S. Army Tropical Research Medical Laboratory, San Juan, P. R.

The influence of crowding on *A. glabratus* was studied as manifested by survival, rate of growth, age at onset of egg-laying and extent of reproduction. Two volumes of water (10 and 200 ml) were employed, and with each a 2-fold variation in snail numbers (1, 2, 4, 8, 16, and 32). All snails used were newly hatched. For a period of 80 days survival was near optimal for as many as 4 snails in 20 ml, and 8 in 200 ml, of water. Mortality was excessive with large numbers. Onset of oviposition was delayed progressively with increased snail numbers. With one snail in each glass (200 ml) mean sizes at 20, 40 and 80 days were 5.3, 10.2 and 13 mm. These figures constituted controls and were essentially identical with results of previous basic studies. With 32 snails in each glass (200 ml) the corresponding figures were reduced about half (2.6, 5.3 and 8.3 mm). With 20 ml of water the results were similar but of a lower order. Egg production during the first 16 days of laying for 1 snail per glass (200 ml) gave mean figures of 5.3 clutches per snail, 33 eggs per clutch and 10.9 eggs per snail per day. With 32 snails in each glass (200 ml) corresponding results were 0.1 clutch, 16.2 eggs per clutch and 0.1 egg per snail per day. Rates of reproduction were less in 20 ml of water. During the second 16-day period, with 1 snail in 200 ml of water, mean figures were 10.5 clutches per snail, 40.2 eggs per clutch, and 26.4 eggs per snail per day while, with the larger numbers of snails, reproduction continued to be greatly reduced. The median ages at which eggs appeared in glasses with 1 and 32 snails, respectively, were 40 and 81 days for 200 ml, and 48 and 86 days for the 20-ml volume.

24. *Streptomycin-induced Inhibition of Growth and Reproduction in* Australorbis glabratus. Eli Chernin, Harvard University, Boston, Mass.

Recent studies, in which a growth-supporting diet was developed for axenic *A. glabratus*, also revealed that streptomycin markedly inhibited growth of these snails. This finding led to investigation of the effects of this antibiotic on snails maintained in aquaria.

The addition of 50 to 100 μg/ml of streptomycin sulfate to aquarium water had 2 effects on snails:

a) elicited an immediate behavioral reaction involving "convulsive" gyrations lasting 2 to 4 days; b) produced a complete, or almost complete, inhibition of growth and reproduction despite resumption of almost "normal" behavior. In one typical 21-day experiment, a control group grew from 6.9 mm to 12.6 mm (means) while a streptomycin-treated lot had increased by only 0.5 mm. In 2 groups (20 snails each) compared for reproductive output during a 21-day period, 221 egg-masses were deposited by the control group and only 1 by the group in water containing 100 μg/ml streptomycin. In these experiments, the inhibitory effects were produced and maintained by a single dose of streptomycin introduced at the start; this and related data showed that streptomycin has a "residual" potency vs. *A. glabratus*. Streptomycin (or dihydrostreptomycin) apparently acts directly upon the snails rather than in an intermediary fashion. The effects of certain inorganic salts in preventing the appearance of the behavioral reaction to streptomycin, and in partially blocking the growth-inhibitory action of the antibiotic, have also been noted. This is believed to be the first report of streptomycin-induced inhibition of growth in a metazoan.

*25. *On the Strain Characteristics of* Schistosoma japonicum *from Two Isolated Endemic Areas in Japan*. H. F. Hsü and S. Y. Li Hsü, State University of Iowa, Iowa City.

Four geographical strains of *S. japonicum*, the Chinese, Formosan, Japanese and Philippine, have been proved to possess distinctive characteristics.

Two Japanese sub-strains, one from Yamanashi on Honshu Island, and one from Kurume on Kyushu Island, were chosen for this study. As the prepatent period and the size and shape of the eggs of this parasite have been shown to be good criteria for strain differentiation, the present studies were made concerning these two characteristics. Albino mice were used as the experimental animals.

The results showed that so far as the prepatent period and the size and shape of the eggs were concerned, there were, in general, no significant differences between these two Japanese sub-strains.

26. *Phagocytosis and Opsonization of Typhus Rickettsiae*. Charles L. Wisseman, Jr., and John R. Gauld, University of Maryland, Baltimore, Md.

The role played by polymorphonuclear and mononuclear phagocytic cells in the defense against, or pathogenesis of, infections caused by the intracellularly parasitic rickettsiae has not been well-defined although scattered observations in the literature suggested that interesting, and perhaps important, interactions occur which may differ significantly as between these two categories of cells. Initial explorations in our laboratory into this general area have begun with studies on the dynamics of phagocytosis of *Rickettsia prowazeki* and *R. mooseri* by mouse polymorphonuclear leukocytes (PMN's) in vitro. Quantitation methods have been devised or adapted which have permitted the following observations: 1) PMN's phagocytize typhus rickettsiae to a small degree which varies with rickettsial concentration. 2) Homologous convalescent human or guinea pig serum markedly increases phagocytosis of the rickettsiae (opsonization). 3) Cross-opsonization occurs, i.e., epidemic-typhus convalescent serum enhances phagocytosis of murine-typhus rickettsiae, and murine-typhus antiserum increases phagocytosis of epidemic-typhus rickettsiae. Cross reactions are of lesser magnitude than reactions in the homologous systems. 4) Analysis of antigens which participate in the opsonization reactions reveals that the erythrocyte sensitizing substance neutralizes the cross-opsonizing capacity of sera and suggests that it plays a major role in the observed cross-reactions. 5) Opsonizing antibody patterns, studied quantitatively in human subjects receiving killed, Cox-type, epidemic-typhus vaccine or living, attenuated, strain-E vaccine of Fox, and in patients experiencing initial or recrudescent epidemic-typhus infections, have been compared with antibody patterns determined by more conventional tests and have been found to be distinctive as well as sensitive indicators of experience with typhus rickettsiae.

* By title.

2:00 p.m., October 31

SYMPOSIUM*

Zoonoses in the Tropics

Moderator, W. H. Wright

27. *Introduction*, W. H. Wright, National Institutes of Health, Washington, D. C.
28. *Some Zoonoses which Constitute Important Health Problems in the Tropics.* James H. Steele, Communicable Disease Center, Atlanta, Ga.
29. *Human and Animal Trypanosomiasis in Africa.* D. G. Davey, Imperial Chemical Industries, Macclesfield, Cheshire, England.
30. *Tropical Bat Rabies as a Public Health and Veterinary Problem.* E. Malaga-Alba, Pan American Sanitary Bureau, Mexico, D.F.
31. *Tropical Arthropod-borne Viruses—Their Importance in Veterinary and Human Medicine.* M. Pollard, University of Texas, Galveston, Texas.

* To be published in the Journal.

9:00 a.m., November 1

(Scientific Session A)

32. *Prevalence of* Toxoplasma *Antibodies in Urban Adults of Memphis, Tennessee.* Colvin L. Gibson and Nell Coleman, National Institutes of Health, Memphis, Tenn.

The prevalence of *Toxoplasma* serum antibodies was determined in 757 adults of Memphis, Tennessee, by means of the Sabin-Feldman dye test. Sera were taken from all Caucasian and Negro applicants for marriage licenses during a 2-month period, and only from Negro applicants for an additional period of several months. Of the total sera tested, 556 were from Caucasians and 201 from Negroes. About 42% were males. Donors were mostly 17 to 40 years of age. Nearly all were urban, but a few were suburban.

The overall prevalence of *Toxoplasma* antibodies (all titers) was 22% in the Caucasian sample and 17% in the Negroes. This difference is not statistically significant. The modal titer for both races was 1:16.

Comparison of the present urban samples with a previously-reported Negro rural population reveals a similar rate of antibody prevalence in the two environments; however, the rural population tended to develop higher titers than did the urban. The possible significance of this finding is discussed.

33. *Results of Dye and Hemagglutination Tests for Toxoplasmosis in a Survey of Trinidad Natives.* Milford N. Lunde and Leon Jacobs, National Institutes of Health, Bethesda, Md.

Dye tests of 121 serum specimens from Trinidad natives revealed an overall prevalence of 52.9% positive at a titer of 1:16 or higher. In the age group of 0–9, 21.4% were positive. Ninety per cent of persons 50 or more years of age were positive. A small group of people of East Indian stock showed no significant differences in prevalence rate. Either these people no longer abide by Hindu proscriptions against meat, or some other method of transmission can occur.

The serums were also tested by the hemagglutination test and the two tests were compared as to agreement. Both tests agreed almost completely as to negativity or positivity. Of 50 serums negative to the dye test, 48 were negative, one had a titer of 1:1 and one of 1:4 by hemagglutination. Of five positive at 1:1 or 1:4 by the dye test, 4 were negative and one positive at 1:4 on hemagglutination. Fifty eight (87.9%) of 66 specimens positive by the dye test at 1:16 or higher gave hemagglutination titers within a 1-tube (4-fold) difference. All specimens agreed within a 16-fold difference in titer.

It appears, therefore, that the hemagglutination test can serve instead of the dye test for epidemiological work on toxoplasmosis.

34. *Toxoplasma Dye-Test Results Obtained by Four Independent Laboratories on Aliquots of 100 Sera.* Marion M. Brooke, Don E. Eyles, Harry A. Feldman, and Leon Jacobs, Communicable Disease Center, Atlanta, National Institutes of Health, Bethesda, Md., and Memphis, Tenn., and State University of New York, Syracuse.

One hundred sera were divided and tested independently by 4 laboratories experienced in the performance of the methylene-blue dye procedure for toxoplasmosis (Sabin and Feldman, 1948).

Identical results were obtained in approximately 40% of the sera tested by all 4 laboratories. In the majority of instances where they were not identical, a 4-fold (1-tube) difference in titer was reported. However, 17% of the disagreements involved at least a 64-fold difference.

35. *The Successful Use of Animal Sera as a Source of Accessory Factor in the Methylene-Blue Dye Test for Toxoplasmosis.* ALEXANDER J. SULZER, MORRIS GOLDMAN AND RUTH H. GOGEL, Communicable Disease Center, Atlanta, Ga.

It has been found that under certain conditions some animal sera may be used as accessory factor sources in the methylene-blue dye test for toxoplasmosis. This can be done in spite of the "nonspecific heat labile modifying factor" reported to be present in animal sera by Sabin and Feldman in 1948. Blood samples of 18 cows were obtained from a local abattoir. Sera from these samples were tested and 2 were found satisfactory as accessory factor sources when used with daily titration of the living antigen.

36. *Daily Titration of Live Antigen to Produce more Uniform Results in the Dye Test for Toxoplasmosis.* MORRIS GOLDMAN, ALEXANDER J. SULZER AND MARION M. BROOKE, Communicable Disease Center, Atlanta, Ga.

Experience in this laboratory has shown that the living *Toxoplasma gondii* used as antigen in the dye test varies in sensitivity to toxoplasma antibody from day to day. In order to compensate for this variation, a method has been developed for titrating the antigen. The results of the titration are used to determine the conditions for setting up the diagnostic test.

Peritoneal exudate from several mice are pooled. Using a small aliquot, tests of 15-minute duration are made using 100% and 80% accessory-factor serum of known activity. From the results of these tests the behavior of the *Toxoplasma* in the pool of exudate can be predicted. By adjusting the concentration of accessory factor and the length of incubation the percentage of satisfactory tests from day to day can be increased significantly.

37. *Methods for the Recovery of* Toxoplasma *from Suspected Animal Hosts.* F. E. JONES, DON E. EYLES AND COLVIN L. GIBSON, National Institutes of Health, Memphis, Tenn.

The availability of a cat population with a very high prevalence of *Toxoplasma* infection made it possible to make a thorough comparison of various techniques used in the recovery of parasites.

Observations showed that intraperitoneal inoculation is much more effective than intracerebral. Most infections were demonstrable during the initial passage in mice; often when mice were sacrificed 1 to 2 weeks after inoculation. The use of dye tests of serum from inoculated mice was an effective method of determining if infection had taken place. Cortisone used in mice receiving inocula resulted in more rapid evolution of the infection but did not increase the number of infections ultimately found.

As a result of the various comparisons, a relatively simple but very effective procedure was developed which is now used routinely. This procedure is described.

38. *The Effect of Metabolites upon the Antitoxoplasmic Action of Pyrimethamine and Sulfadiazine.* DON E. EYLES AND NELL COLEMAN, National Institutes of Health, Memphis, Tenn.

The effect of pyrimethamine and sulfadiazine upon the proliferating forms of *Toxoplasma* is believed due to the imposing of sequential blocks upon the metabolic pathway involving *p*-aminobenzoic acid, folic acid and folinic acid. For this reason it is important to determine the effect of these metabolites upon the action of the drugs, both alone and in combination.

Experiments using acute infections in mice indicated that *p*-aminobenzoic acid significantly inhibits the action of both pyrimethamine and sulfadiazine. When the drugs are used in combination this metabolite has a measurable but not great effect upon the drug action. Folic acid significantly interferes with the action of sulfadiazine but not with that of pyrimethamine. Folic acid has practically no effect upon the drugs given in combination. Experiments with folinic acid are still in progress.

The significance of these observations, and the observations of others on the same subject, in the treatment of human toxoplasmosis are discussed.

39. *The Treatment of Chronic* Toxoplasma *Infections in Mice.* DON E. EYLES AND NELL COLEMAN, National Institutes of Health, Memphis, Tenn.

Previous work has shown that pyrimethamine and sulfonamides alone and in combination have definite chemotherapeutic activity against acute *Toxoplasma* infection in mice and other animals, but there has existed only indirect evidence as to their effect in chronic infections.

Direct observations have been made of the effect of pyrimethamine and sulfadiazine through the use of a strain of *Toxoplasma* which produces chronic infections in mice characterized by large numbers of pseudocysts. When treatment of 2 to 6 weeks duration was given to mice with infections of several months standing, no effect was noted upon the number of pseudocysts or upon the infectivity of the pseudocysts to mice. Observations were also made of the effect upon serologic findings, and another experiment was designed to

determine at what point in the infection forms resistant to treatment appeared.

The significance of the findings in the chemotherapy of this infection is discussed.

40. *Antagonism of the Toxoplasmicidal Activity of Pyrimethamine.* M. KATHERINE COOK, National Institutes of Health, Bethesda, Maryland.

Folic and folinic acids, PABA, and various purines and pyrimidines have been tested as possible antagonists to the activity of pyrimethamine against *Toxoplasma* in tissue culture. Mouse mince-tissue cultures were used for most of the experiments. Of the purines and pyrimidines tested, only thymine showed any antagonistic activity to the toxoplasmicidal activity of pyrimethamine in the tissue-culture system, and the antagonism was only slight. The deoxyriboside, thymidine, and para-aminobenzoic acid showed antagonistic activity, as did folic and folinic acids. Adenine, guanine, cytosine, uracil and xanthine showed no antagonistic activity. Although quantitative data have not been obtained, these antagonism experiments suggest that pyrimethamine acts by interfering with various reactions involved in the synthesis of nucleic acids in *Toxoplasma*. It has been demonstrated that folinic acid is superior to folic acid in the ability to antagonize pyrimethamine. Monkey-kidney cells were unsatisfactory for the direct demonstration of drug antagonism but a technique which used the reversal of drug storage as an indicator of antagonistic activity suggests that this type of system may be of use in future investigations.

41. *The Effect of Pyrimethamine on* Toxoplasma *and Storage of the Drug in Cells in vitro.* M. KATHERINE COOK AND LEON JACOBS, National Institutes of Health, Bethesda, Md.

Growth of *Toxoplasma* in cultures of monkey-kidney epithelium was slightly inhibited by concentrations of pyrimethamine as low as 0.005 mg %. Clearing the parasites from cultures was effected by 3- or 4-day exposures to 0.050 mg % and 0.025 mg %, respectively, when drugging was started 4 hours after inoculation. The drug had no demonstrable effect on nonproliferating toxoplasmas or on the ability of the parasites to enter cells.

Cultures cleared of *Toxoplasma* by pyrimethamine and then washed and incubated in drug-free medium showed delay in lysis time when reinfected with the parasite. This was shown to be due to storage of drug in the cells. On the basis of results of inoculations of tissue cultures into mice, as well as demonstration of a delay in lysis of these cultures following infection with *Toxoplasma*, storage of drug was demonstrable for 4 to 14 days when cultures were incubated in nutrient medium containing 0.15 mg % to 0.50 mg % pyrimethamine, then washed and renewed with undrugged medium.

Monkey kidney, chick-embryo-heart explants, HeLa and human intestinal cells all showed the storage phenomenon. Mouse-embryo mince and KB cultures showed less storage, and human conjunctival epithelium showed none. Age of cells in culture had little effect on ability to store drug.

Storage of drug in monkey-kidney cells 1 week after termination of treatment with 0.05 mg % was confirmed by a microbiological assay procedure on sonic extracts of the cells.

*42. *The Development of a Pyrimethamine-resistant Line of* Toxoplasma. M. KATHERINE COOK, National Institutes of Health, Bethesda, Md.

The development of pyrimethamine-resistant lines of *Toxoplasma* has not been investigated previously, but may be of some importance in the treatment of clinical toxoplasmosis. Drug-resistant lines of *Toxoplasma* may also be useful in studies of metabolic pathways present in the parasite. It was found that lines of *Toxoplasma* 64 × as resistant to pyrimethamine as the original line, may be easily developed in tissue culture by exposing toxoplasmas to increasing concentrations of sublethal doses of pyrimethamine and allowing a "rest period" in mice between the tissue-culture transfers. These lines of parasites maintained their drug-resistant properties when cultured without the drug for at least 3 months as shown in in vitro tests, but did not show pyrimethamine-resistance when tested in mice, probably due to differences in the metabolic handling of the drug in vitro and in vivo. Other experiments to determine the minimum effective dose of pyrimethamine showed that occasional cultures were positive for toxoplasma when high concentrations of, and long exposures to, the drug were employed. Subsequent tests of these parasites for pyrimethamine-resistance were negative, indicating that mutation leading to drug resistance had not taken place. Parasites which were grown continuously in the presence of pyrimethamine for month periods showed that pyrimethamine-requiring lines could not be produced.

* Read by title.

9:00 a.m., November 1

(Scientific Session B)

43. *Histological Studies of Cecal Tissue of Guinea Pigs Fed a Purified Ration and Used for Experimental Amebiasis.* D. JANE TAYLOR AND ROBERT T. HABERMANN, National Institutes of Health, Bethesda, Md.

The Roine and Elvehjem (R-E) purified diet for guinea pigs has been shown to enhance the susceptibility of this animal as a host for *Entamoeba histolytica* (Taylor and Greenberg, Fed. Proc., 10: 396, 1951). Lynch and Dardin (Am. J. Trop. Med. Hyg., 6: 395, 1957) reported that the same diet in guinea pigs caused histological changes in the cecum as early as 14 days after their animals were placed on this ration. Since this diet has served in our laboratory as a basis for experimental work on the chemotherapy of amebiasis it was considered necessary to our program and to basic knowledge on the amebic infection to determine if the Roine and Elvehjem dietary regimen was initiating similar changes in the guinea pigs in our studies.

On the R-E diet our young guinea pigs have been reared to maturity and mated, and have given birth to healthy offspring which in turn were raised to adulthood. Also, over the years numerous uninoculated control animals, as well as test guinea pigs which did not become infected following inoculation with amebae, have been kept for as long as 5 weeks and have been found to gain weight and to appear normal in every respect.

Weight records and histological observations will be presented on guinea pigs of the NIH strain fed purified diet, and also on those given a regular laboratory chow supplemented daily with kale for intervals up to 4 weeks.

44. *Studies on the Growth Requirements of* Entamoeba histolytica. *The Ingestion of Altered Bacterial Structures by* E. histolytica *in the Shaffer-Frye Medium.* JAMES G. SHAFFER, ROGER W. SCHULER AND IRIS D. KEY, Chicago Medical School, Chicago, Ill.

Attempts to demonstrate the ingestion of bacterial cells by *E. histolytica* in the Shaffer-Frye (S-F) medium have resulted in the observation that the gram-negative, anaerobic streptobacillus used in the medium undergoes morphologic changes due to the action of the penicillin G. These altered structures are ingested by the propagating amebas in the S-F medium. No typical bacterial cells have ever been demonstrated in the amebas.

Periodic observations of the bacterial cells in the S-F medium by darkfield microscopy have revealed that after 10 to 12 hours there appear bubble-like protrusions on the cell wall. These enlarge to a diameter about one-half the length of the bacterial cell and then break off and float free in the medium as a round body. The number of these round bodies increases markedly during the succeeding 12 to 18 hours and, after 24 hours, predominate in the medium.

The amebas have been observed ingesting the round bodies in numbers roughly proportional to their concentration. Six variants of the streptobacillus have been studied. They vary in the production of round bodies and this variation has been found to be related to the ability of the variants to support propagation of *E. histolytica*. Thus, there is suggestive evidence that ingestion of the round bodies is related to propagation of the amebas in the S-F medium.

45. *Cultivation of* Entamoeba invadens *axenically in the absence of tissue.* NORMAN R. STOLL, The Rockefeller Institute for Medical Research, New York.

A strain of *E. invadens* (from Dr. E. Meerovitch, Institute of Parasitology, MacDonald College, P. Q.) received in serum-saline containing a piece of sterile liver, has been maintained contaminant-free in serial transfer in fluid cultures without tissue. In one line, in a period of 12 months, 17 consecutive subcultures made at intervals of 4 to 55 days, averaged 22 days between transfers.

The basic medium consists of infusion broth supplemented with acidified raw-liver extract (RLE). The former is preferably made from liver (LB) (although beefheart may substitute) and is autoclaved. RLE is prepared cold and sterilized by Seitz filtration (Stoll, 1953). Growth is favored by inclusion of a moiety of peptone or trypticase. Worked enhancement in yield and survival of amebae occurs with addition of autoclaved mucin (1701-W); somewhat in relation to the amount introduced. (May this avidity for mucin suggest the natural conditions in the parasitized gut?)

No antibiotics are employed in the cultures. Using 16 x 150-mm tubes, vaseline-sealed with 5 to 8 ml medium, survival of vegetative forms has permitted successful subinoculation of individual cultures after 11 months. Screw-cap tubes of the same size without other seal and with 15 ml of medium have been alternatively used with good, though not always excellent, growth and survival. Commercial frozen chicken livers have been one satisfactory source for both LB and RLE.

46. *Acute African Amebiasis. Clinical-Laboratory Studies and Comments on Response to MA-307.* HAMILTON H. ANDERSON, ALEXANDER J.

WILMOT AND LEONARD FREEDMAN, Natal University School of Medicine, Durban, S. Africa, and University of California, San Francisco, Cal.

Twenty-one Bantus exhibiting signs and symptoms of acute dysentery were subjected to a battery of liver-function tests, studies of the electrophoretic pattern, kidney-function tests, complement-fixation studies, and biopsies of liver (3) and sigmoid ulcers. The history, physical findings and conventional laboratory examinations, including iron-hematoxylin staining of fecal and proctoscopic specimens, were correlated with other observations.

Ten of the patients were treated with MA-307 (N - N' - dipiperonyl - 5 - 11 - diaminopentadecane) given orally in 100-mg amounts thrice daily over 10 days in hospital. Proctoscopic examinations were made at 5-day intervals, and daily stool specimens were taken to follow progress of therapy in each patient. In those not responding to MA-307 alone, emetine was given in 0.65 gm total dose over 10 days.

47. *Mass Ambulatory Treatment of Amebiasis, With Observations on Acquisition of New Infections in an Area of High Endemicity.* DALE E. WYKOFF, LAWRENCE S. RITCHIE AND LYMAN P. FRICK, Walter Reed Army Institute of Research, Washington, D. C.

In February, 1952, 235 persons in a group of approximately 580 from two rural Japanese villages were treated for intestinal protozoan infections with carbarsone (1 tablet t.i.d. for 7 days) and chiniofon (3 tablets t.i.d. for 7 days) in sequence. Efficacy of treatment and acquisition of new infections were determined from a series of single and multiple-stool examinations performed periodically on all individuals during the next 2 years.

Therapeutic indices established 6 months after treatment for the number of infections indicated were as follows: 117 *Endamoeba histolytica*, 96%; 201 *E. coli*, 82%; 164 *Endolimax nana*, 67%; 30 *Iodamoeba bütschlii*, 100%; and 39 *Giardia lamblia*, 44%. Treatment reduced original rates of amebiasis in the 2 villages from 14% and 29% to about 2%. After a year the rates had increased to 3% and 7%.

On the basis of data obtained from 3 series of examinations performed during the first year after treatment, about 1% of persons in 1 village and 4% in the other acquired new infections with *E. histolytica* during that year. All cases appeared to have originated from outside the family. Acquisition rates for other infections were: *E. coli*, 15% and 18%; *E. nana*, 4% and 17%; and *G. lamblia*, 7% and 18%. These differences between respective rates of acquisition tended to confirm certain epidemiologic observations made previously in the area.

In general, as the proportion of family members receiving therapy increased, fewer new infections occurred in the family. However, rates did not differ significantly.

48. *The Morphological Details of* Nocardia *as Seen with the Electron Microscope.* MARGARET HOTCHKISS, AND O. F. EDWARDS, University of Kentucky, Lexington, Ky.

For taxonomic purposes in the separation of the genus *Nocardia* from the genus *Streptomyces* the permanence of the mycelium of the *Streptomyces* has been stressed. However, among the members of *Nocardia* there is great variation in degree of permanence; some members readily fragmenting and others remaining adherent to the medium and even producing minute aerial hyphae. It has been stated that the *Nocardia* do not cut off conidia, and yet in such species as *Nocardia asteroides* round, coccoid bodies are very generally observed in microscopic preparations. The electron microscope was used in this study to elucidate some of these morphological details.

The electronographs indicated that the filaments produced true branching. There was variation in electron density in different parts of the filaments, and in some cases an electron-dense band was seen across the filament. A wall of definite rigidity was seen at the periphery of filaments. The growing tip of the primary and lateral filaments were swollen, and exhibited uniform electron density, but older filaments showed aggregations of electron-dense material in distinct clumps. Some electronographs of the coccoid bodies were obtained, and these had a central core of electron-dense material which was more ovoid than spherical. As yet, electronographs showing the origin and development of these bodies have not been obtained.

49. *The* Salmonella *Infection Rate in Egyptian Villages.* C. D. MCGUIRE AND T. M. FLOYD, Naval Medical Research Institute, Bethesda, Md.

Salmonella infections are among the most prevalent bacterial diseases of Egyptian village inhabitants. In order to ascertain if a variation in the incidence of *Salmonella* infections occurs as a function of village economic and environmental conditions a *Salmonella* survey was conducted in 3 Egyptian villages of similar population. The villages selected were differentiated by the nature of the inhabitant's employments, average income levels, and existing sanitary facilities.

A random sample of urine and feces was collected from the inhabitants of each village. From a total population of 14,855 persons, 4,235 specimens of urine and feces were analyzed. The survey

was conducted during the winter months in order to minimize the influence of summer epidemic conditions.

The results of the study showed an average *Salmonella* infection rate of 13 per M, of which 8 per M were *S. typhi*. No significant difference in the incident *Salmonella* infection rate was observed to occur between the villages studied.

The study indicated that income level, nature of employment, and village sanitary conditions did not markedly influence the *Salmonella* infection incidence in Egyptian village populations.

*50. *Studies in Shigellosis. VII. Observations on the Transmission of* Shigella *in an Egyptian Village.* THOMAS M. FLOYD, Naval Medical Research Institute, Bethesda, Md.

Feces, flies, fingers and food are generally accepted as the customary vehicles for transmission of *Shigella*. However, many of the supporting data have been circumstantial or obtained during epidemics of shigellosis, and few direct observations have been made during nonepidemic conditions. The high endemicity of bacillary dysentery in an Egyptian village provided an excellent opportunity to make such observations.

The presence of infected feces in the environment was indicated by the prevalence of *Shigella* infections and the defecation habits of the villagers. During spring or fall as many as 25% of the children were shown at one time to have been excreting *Shigella*.

Houseflies were trapped in and around villages and pools of 25 flies were cultured for enteric organisms. Twelve (7.6%) of 156 pools contained *Shigella*.

Cultures were made from hands of mothers of children with bacillary dysentery. As many as 5% of these cultures were positive for *Shigella*. Concurrent infections were noted frequently among siblings.

Foods of various types were cultured for enteric pathogens. Coliform counts indicated universal contaminations with feces. *Shigella* were recovered frequently from fresh meat and fish and rarely, or not at all, from vegetables, eggs, bread and milk products. *Shigella* were not recovered from highly polluted well water.

51. *Studies in Filariasis. I. The Behaviour of Microfilariae of* Dirofilaria immitis *in The Wax Moth Larva* (Galleria mellonella). MEIR YOELI, NELDA ALGER AND HARRY MOST, New York University-Bellevue Medical Center, New York, N. Y.

Heparinized blood containing large numbers (1100–1200 per 20 mm³) of microfilariae of *D. immitis* was experimentally injected into mature larvae of *G. mellonella*. Development of microfilariae of *D. immitis* at room temperature (27°C–30°C) was observed in the hemolymph of the *Galleria* larvae, as well as near the fat body and the adjacent tissues. Live, slowly moving "sausage forms" were seen on the fourth and subsequent days after inoculation. Development appears to be halted at the "sausage" stage or at the second developmental phase. This corresponds to developmental forms of 5 or 6 days in susceptible mosquito vectors. No preinfective or infective forms were detected in 12 experiments carried out to date.

A study of cellular organization of the growing microfilaria of *D. immitis* under the above-mentioned experimental conditions was made from fresh material, stained smears and histological sections.

Cellular reactions such as encapsulation of microfilariae, pseudotubercle and giant-cell formation and chitinization similar to those encountered in the tissues of *D. immitis*-infected mosquitoes were observed in infected *G. mellonella* larvae.

In *G. mellonella larvae* injected with *D. immitis* and kept at 37°C, no metamorphosis of microfilariae was observed, although the microfilariae remained alive for periods of up to 11 days.

The stages of development of *D. immitis* in *G. mellonella*, an experimental nonspecific host, will be shown. The importance of these findings will be discussed in the light of future possible experimental growth of microfilariae outside their insect vectors.

52. *The Persistence of Acquired Immunity to the Filarial Worms of the Cotton Rat.* ETTA MAE MACDONALD AND J. ALLEN SCOTT, University of Texas, Galveston, Texas.

We have reported previously that when cotton rats are given a superimposed infection of *Litomosoides carinii* 30 days after a primary infection, the worms of the superimposed infection are retarded in growth and development as compared to worms of parallel primary infections. The present experiments were designed to determine how long this immunity persists in the absence of reinfection. Cotton rats which had received original infections at the age of 2 to 12 months were given challenging infections 10 to 18 months later. In the animals which still harbored some live worms, the growth of the challenging infection was retarded as in the previous short term experiments. When only dead worms were found at autopsy, little or no retardation of growth of the worms of the challenging infections was found.

53. Withdrawn.

* Read by title.

2:00 p.m., November 1

54. *Brown Spider Bites in the Midwest.* W. A. Sodeman, Curtis W. Wingo, James A. Atkins and Joseph E. Flynn, Jefferson Medical College, Philadelphia, Pa., and University of Missouri, Columbia, Mo.

Spider bites due to *Loxosceles* are little known in the United States. The clinical picture is a distinctive one quite unlike that due to the black widow and tarantula bite. Severe pain develops locally at the site of the bite and, within the next 24 to 36 hours, a systemic response is likely to occur. This is characterized by restlessness, fever, and at times the development of a generalized, scarlatiniform eruption. At the site of the bite there develops an ischemic area surrounded by redness and extravasated blood. The white area progresses into a rapid necrosis, forming a deep ulcer with sharp, ragged edges and a yellowish white base, at times exposing the underlying muscles. Slow, gradual evolution with scarring appears.

Kodachrome slides of the development of the lesion and of the generalized eruption have been made in a number of instances. They will be used to demonstrate the progress and the clinical picture. Identification of the spider will be discussed. Studies of the toxin, its specificity, and its nature will be cited. Experimental production of lesions in animals will be demonstrated. It will also be demonstrated that this spider, undescribed in most medical texts in this country, is responsible for a dramatic clinical picture not uncommon in Central Missouri.

55. *Tests with Compounds Showing Synergistic Action with Rotenone Against Body Lice.* M. M. Cole and P. H. Clark, U. S. Department of Agriculture, Orlando, Fla.

Sulfoxide and piperonyl butoxide were highly synergistic with rotenone in laboratory tests against body lice, and 1,2-methylenedioxy-4-[2-(octylsulfonyl)propyl]benzene, 5-butyl-5-ethyl-2-(3,4-methylenedioxyphenyl)-*m*-dioxane, and the *alpha*-allylpiperonyl ester of fencholic acid showed a lower degree of synergism. Rotenone alone was not lethal to lice at concentrations up to 5%, while the other compounds alone caused high mortality at this concentration. The effectiveness of mixtures varied with the concentration of both sulfoxide and rotenone.

A powder containing 0.5% of rotenone and 5% of sulfoxide was highly effective for a longer period than one containing 0.2% of pyrethrins and 2% of sulfoxide in tests against DDT-resistant lice on human subjects.

56. *The Measurement of Adult Populations of the Eye Gnat,* Hippelates pusio *Loew.* Richard P. Dow, Communicable Disease Center, Logan, Utah.

Hippelates pusio, a probable vector of bacterial conjunctivitis, is attracted to the skin and hair of various mammals including man. It is especially annoying about the eye, where it is more frequent when the eyes show signs of infection. Traps baited with putrefied liver, which are used to measure the prevalence of *H. pusio,* attract also *Hippelates bishoppi,* a closely related species which is not attracted to mammals. *H. bishoppi* may exceed 50% of a trap collection but not when the trap is located near an animal enclosure such as a chicken pen. Weights of dried trap collections are used to calculate population index. An alternate method of estimating vectoring potential is provided by counting gnats attracted to eyes of children.

57. *Problems and Techniques in the Colonization and Laboratory Study of the* Culex (Culex) *Vectors of the Encephalitides.* Herbert C. Barnett and Douglas J. Gould, U. S. Army Med. Research Unit, Kuala Lumpur, Malaya, and Walter Reed Army Institute of Research, Washington, D. C.

Available evidence suggests that the arthropod-borne encephalitides are carried principally by mosquito species of the banded-proboscis group of the subgenus *Culex (Culex).* Studies on these mosquitoes have been hampered by difficulties in colonizing and maintaining them under laboratory conditions. Recent colonization of several vector or suspected-vector species including *C. tarsalis, C. tritaeniorhynchus,* and *C. gelidus* has provided the basis for a rational approach to the colonization and laboratory rearing of the entire subgenus.

Colonization is favored by the introduction of very large volumes of stock mosquitoes, to permit mating of those specimens which are best capable of reproducing under the conditions provided. This selection process is enhanced by proper lighting conditions, which appear to be of prime importance. Either natural or simulated dawn and sunset lighting intensities have been shown to be necessary for copulation and mating. Similarly, proper length of photoperiod (daylight) has also proven to be a basic requirement for the mating of some species.

The question of the extent to which laboratory colonization affects the biology of a species was studied during the course of the colonization of *C. gelidus.* Each laboratory generation was maintained separately, thus permitting comparison of the biologies of the wild forms and the forms of the first 5 generations reared in the laboratory. Gross

changes in reproductive biology were noted during the first 4 generations but other biological changes could not be detected.

*58. *Feeding* Aedes aegypti (L) *through Animal Derived Membranes*. I. BARRY TARSHIS, Fort Detrick, Md.

An apparatus and a technique for in vitro feeding of *Aedes aegypti* are described. The apparatus consists of a thermostatically controlled water bath, a container to hold the blood, and a feeding cup with a chamber to hold the mosquitoes and a membrane through which they can feed.

The following conditions were found to be optimum for feeding these mosquitoes through membranes: (1) thawed, heparinized beef blood; (2) a blood temperature of 34°C; (3) an ambient temperature of approximately 26°C; (4) a thin, animal-derived membrane; (5) mosquitoes 4 to 7 days of age and deprived of sugar solution 24 hours prior to the blood meal; (6) feeding during the morning hours; and (7) a feeding period of 180 minutes.

Of 14 membranes screened in the current investigations, Nearkid, Fourex, Silverlight, hamster skin, and rabbit skin gave the highest feeding rates with these mosquitoes.

This apparatus and technique should have a wide range of application. This type of technique has potentialities in basic entomological research on problems such as transmission of bacteria, viruses and protozoa; physiology and nutrition; chemotherapeutic control of ectoparasites; repellency studies; and behavior.

59. *Urban Dispersal and Activity of* Aedes Aegypti. HARVEY B. MORLAN, RICHARD O. HAYES AND H. F. SCHOOF. Communicable Disease Center, Savannah, Ga.

In an urban area of Savannah, Ga., 4.7% of 9,615 *Aedes aegypti* females released in 10 tests were recovered.

In five dispersal tests in which collection was limited to one block (within a radius of 275 feet), 78% of the recovered mosquitoes were within 75 feet of the release point; 18% and 4% were recovered at distances of 76 to 175 feet and 176 to 275 feet, respectively.

In a release of 1,200 females in which collection was extended to an area within a radius of 575 feet, 25% of the recovered mosquitoes were collected within 75 feet of the release point, 36% at a distance of 76 to 175 feet, 14% at 176 to 275 feet, 18% at 276 to 375 feet, 2% at 376 to 475 feet and 5% at 476 to 575 feet.

In multiple-point releases of 400, 900 and 1,200 females in a single block, attack rates (number of biting records divided by the number of residents in cooperating households) were 57, 90 and 187, respectively.

60. *Organophosphorus Compounds as Residual Treatments for Adult Mosquito Control*. W. MATHIS AND H. F. SCHOOF, Communicable Disease Center, Savannah, Ga.

Laboratory tests with commercially available organophosphorus and chlorinated hydrocarbon insecticides alone and in various combinations have been evaluated against susceptible or dieldrin-resistant *Anopheles quadrimaculatus* adults and susceptible or DDT-resistant *Aedes aegypti*. Malathion and Parathion were the two most promising organophosphorus compounds when used alone or in combination with DDT or dieldrin. EPN showed some promise when used in combination with DDT.

Promising formulations were tested in animal bait (rabbit) stations. Mosquitoes were placed so that they had the choice of entering the treated bait station or remaining in the entrance cage. Results are given on the mortalities obtained and numbers that were able to obtain a blood meal and survive for 24 hours.

61. *A Field Method for Detecting Resistance in Adult Mosquitoes*. W. MATHIS, R. W. FAY AND H. F. SCHOOF, Communicable Disease Center, Savannah, Ga.

A field kit for use in determining susceptibility levels in adult mosquitoes has been devised. Modified plastic tubes (5″ x 1¾″ diam.) are used as collecting, holding, and testing chambers. Test chambers are lined with paper commercially impregnated with either DDT or dieldrin. Results are given on mortalities obtained with susceptible and dieldrin-resistant strains of *Anopheles quadrimaculatus* and DDT-resistant and susceptible strains of *Aedes aegypti* exposed for different periods at different concentrations of each insecticide. Results are also given on the effect of continuous use of treated papers and their shelf life.

62. *Jungle Yellow Fever in the Magdalena Valley, Columbia*. HERNANDO GROOT AND AUGUSTO GAST-GALVIS, Instituto Carlos Finlay, Bogota, Colombia.

During the period 1934 to 1957, 369 cases of jungle yellow fever have been diagnosed, by postmortem liver examination, among the residents of the Magdalena Valley and the foothills of the neighboring cordileras. The majority of the cases were from the foothills, where the density of population is much higher than in the sparsely populated valley. Most of the Magdalena River valley is covered by dense jungle which extends from the river up to the mountains. In the foothills, mainly an agricultural region, the forest areas are smaller.

The highest incidence of cases was observed in

* By title.

the months of December and January, which coincides with the end of the rainy season. Further, a study of the monthly incidence of cases throughout the 23 years of observation shows that there is some periodicity in their appearance, apparently unrelated to precipitation. This picture presents itself especially in individual localities.

In Muzo and San Vicente, 2 places in this region about 170 km apart, 7 distinct periods of yellow-fever activity have been manifest, (judging from qualitative viscerotomy data) alternating with periods of inactivity. The duration of the active periods has ranged from 8 to 27 months in Muzo, and from 1 to 12 months in San Vicente; that of the inactive periods from 13 to 31 months in Muzo and from 19 to 43 months in San Vicente.

As a preliminary step in the study of the epidemiology of yellow fever in a representative locality of both the valley and the foothills, a serological survey (neutralization and hemagglutination tests for yellow fever and other viruses) was carried out in San Vicente with the following results: high immunity rates against yellow fever in residents of both the foothills and the valley, influenced undoubtedly by the previous vaccination campaigns; low immunity rates for dengue, Ilheus and Venezuelan equine encephalitis viruses among the residents of the foothills; and high immunity rates for those same viruses among the valley inhabitants; which would indicate a greater activity of these viruses in the valley.

63. *Electron Microscopy of Psittacosis Virus* in situ *in Tissue Culture*. Samuel D. Bell, Jr., and Leon P. Weiss, Harvard University, Boston, Mass.

From a continuously cultivated cell line of HEP-2 cells, roller-tube cultures containing approximately 100,000 to 500,000 cells in one ml of medium were inoculated with 100 to 1000 infectious particles of the 6 BC strain of psittacosis. At the conclusion of the experiment, 150 hours after inoculation, there was an increase in titer of approximately 5 logs.

For microscopic study, cells were fixed and brought into methacrylate *in situ*. The fixed cells were then scraped from the wall of the tubes, lightly centrifuged, and embedded in gelatin capsules. Sections were cut for light and electron microscopy. In addition, celloidin whole mounts were made of comparable cultures.

At 150 hours many foci of degenerating cells were present in experimental cultures, although mitotic forms were also frequent. At this time in Giemsa-stained preparations approximately half the cells in these cultures contained inclusions characteristic of psittacosis. In electron micrographs the ground cytoplasm of these cells was denser than that of the controls and their mitochondria were fewer, shorter and less branched.

The elementary bodies were concentrated in the paranuclear region, or were dispersed throughout the cytoplasm in no apparent relation to cellular structures. The characteristic form of the elementary bodies was oval, measuring approximately 0.25 x 0.20 μ, and consisted of a thin membrane or lamellae enclosing relatively dense, closely packed, granular material. The paranuclear formations consisted, typically, of elementary bodies and vesicles of approximately the same dimensions as the elementary bodies. The vesicular component appeared derived from the endoplasmic reticulum or the Golgi complex of the host cell. The viral and cytoplasmic elements tended to form a mosaic pattern in which an elementary body was surrounded by vesicles. These paranuclear formations were identified in serial sections cut for the light microscope, as typical of the inclusions of psittacosis.

64. *Fractionation and Serologic Evaluation of* Trypanosoma cruzi *Antigens*. Earl H. Fife, Jr. and John F. Kent, Walter Reed Army Institute of Research, Washington, D. C.

Dried *T. cruzi* were treated with anhydrous ether to remove lipids responsible for cross-reactions with syphilitic sera, and the residue analyzed for specifically-reactive components. Material extracted with buffered saline solutions in the pH range 6.2 to 9.6 exhibited maximal specific reactivity. A fraction obtained at pH 7.3 to 7.4 was studied by physico-chemical methods. Previous investigators had extracted a specifically-reactive carbohydrate antigen, but no apparent consideration had been given to possible protein components. In the present study the essentially lipid-free antigen was treated with chloroform under conditions considered to minimize antigen deterioration. A fraction isolated as a chloroform "gel" was identified as protein, the remaining water-soluble fraction as carbohydrate. Both fractions exhibited specific serologic activity of high titer, and were essentially free of anticomplementary activity. The sensitivity and specificity of the fractions were evaluated in complement-fixation tests with human sera representing homologous and heterologous infections. The findings are discussed from the point of view of their application in the diagnosis of American trypanosomiasis.

65. *The Cultivation of* Trypanosoma cruzi *in HeLa Cell Culture*. R. G. Yaeger, Tulane University, New Orleans, La.

Blood-stream forms of *T. cruzi*, Brazil strain,

were isolated from the blood of infected mice by a combination of centrifugation and agglutination with antiserum for mouse blood cells, and introduced into 3-day-old HeLa cell cultures which were maintained in a medium consisting of Hank's solution and human or bovine serum. Penicillin and streptomycin were usually added to the medium to control bacterial contamination. Cultures were grown in screw-capped milk-dilution bottles at 37°C.

Intracellular leishmanian forms were seen on the third day after inoculation. Extracellular trypanosomes were present at all times, at first from the original inoculum and later by rupture of the infected HeLa cells after maturation from the leishmanian form. Examination of the living cultures on the fifth day revealed some cells filled with highly motile parasites which were either spindle-shaped or trypanosomal in form. Trypanosomes were observed to escape from ruptured host cells.

Giemsa-stained smears of the culture material showed that 5 to 10% of the HeLa cells were infected; some of the larger cells contained 200 or more parasites, and multinucleate as well as uninucleate cells were infected. In cultures which were 10 or more days old, cells containing early leishmanian forms, intermediate forms or trypanosomal forms were present simultaneously.

66. *African Field and Laboratory Studies on the Cultivation of Sleeping Sickness Trypanosomes from Patients and Suspects.* DAVID WEINMAN, Yale University, New Haven, Conn.

In East Africa (Uganda) where these investigations were carried out, the human trypanosomes are readily grown by a method previously described by the author. Both *T. rhodesiense* and *T. gambiense* were isolated repeatedly from the blood and with approximately equal ease from the cerebro-spinal fluid. Isolations were obtained not only from proved cases, but also from suspects reported as "negative" when tested by conventional methods. The cultural method also revealed trypanosomal infection of the cerebro-spinal fluid which was not otherwise detected at the time, and before the cell count and the protein content had passed normal limits.

The cultural method appears to be more sensitive in certain respects than the conventional method, but it is not so rapid. It will not detect all infections; on the other hand it will uncover many which would otherwise be ignored.

At present, the very best method of diagnosis will combine the conventional methods and the cultural method. This may not be practical for routine diagnostic work, but when difficulties in diagnosis arise, in certain types of control work, and above all in research investigations, the clinician, the epidemiologist and the experimentalist will find that the cultural method offers essential information which may otherwise be unobtainable.

67. *Electrocardiographic Studies in Experimental Chagas' Disease in Mice* (Mus musculus). A. PACKCHANIAN AND A. ROBINSON, University of Texas, Galveston, Texas.

A modified electrocardiographic assembly with string galvanometer having a camera speed 90 mm per second yielded excellent electrocardiograms of normal mice and mice infected with *Trypanosoma cruzi*.

Electrocardiograms were taken of 70 mice infected with *T. cruzi* at various stages of the disease. The mean values of ECG in acute, subacute, and chronic phases of the disease were determined. Statistically significant differences of means between infected and uninfected animals were found in measurements of P-R, QRS, and QT intervals and in the heart rate.

2:00 p.m., November 1

(Motion Picture Session)

68. *The Control of Non-periodic Filariasis in the South Pacific.* JOHN F. KESSEL, University of California at Los Angeles and Institut de Recherches Medicales de l'Oceanie Francaise, Papette, Tahiti.
69. *Primitive Groups of Indians and Djukas in Suriman, Visited on a Canoe Trip to the Brazilian Border.* HAROLD W. BROWN, Columbia University, New York, N. Y.
70. *Medicine in the Tropics.* American Foundation for Tropical Medicine, New York, N. Y.
71. *The Story of Antricide.* D. G. DAVEY, Imperial Chemical Industries, Ltd., Macclesfield, Cheshire, England.

71a. *Tropical Diseases in Africa.* THOMAS A. BURCH, National Institutes of Health, Bethesda, Md.

9:30 a.m., November 2

72. *Broadened Neutralizing-antibody Spectrum Induced by 17-D Yellow Fever Vaccine in Human Subjects Previously Infected with Japanese Encephalitis Virus.* BENJAMIN H. SWEET AND CHARLES L. WISSEMAN, JR., University of Maryland, Baltimore, Md.

The increasing multiplicity of group-B Arbor viruses potentially important as causes of human disease calls for elucidation of basic principles upon which simplified, practical, immunization regimens may be built. Our group has been studying cross-immunity patterns induced in human subjects by selected combinations of both living attenuated and killed group-B virus vaccines and the influence of previous experience with heterologous group-B viruses on the efficacy of these vaccines.

The present report deals with two groups of medical-student volunteers, one from Baltimore (no previous experience with group-B viruses) and one from the Tokyo area of Japan (all with previous infection with Japanese encephalitis (JE) virus) who were inoculated with 17-D yellow fever (YF) vaccine. Observations made over a 6-week period indicated that previous JE infection distinctly influenced responses to YF vaccine. The Baltimore group developed high titers of neutralizing antibodies which reacted significantly only with YF virus. In contrast, volunteers with pre-existing JE antibodies developed a different neutralizing antibody pattern: maximal titers of YF antibodies were significantly lower, yet the spectrum of group-B antibodies produced was considerably broader, i.e., cross-reactions with other group-B viruses appeared. For example, all of the Japanese volunteers developed dengue-1 neutralizing antibodies and some developed dengue-2 antibodies even though presumably none had had previous dengue infection. Moreover, some developed transient increases in JE neutralizing antibodies over prevaccination levels.

Preliminary results point to a lesser 17-D viremia in the Japanese group. However, no difference in time of antibody appearance was detectable between the two groups.

These findings are compatible with the idea that a simplified immunization regimen might employ cross-reactions to broaden the protection afforded and hint that cross-reactions may limit the use of living vaccines as multiplicity of group-B experiences increases.

73. *Induced Virus Infections in Men with Mengo, Bwamba, and Semliki Forest Viruses.* CHESTER H. SOUTHAM, Memorial Center for Cancer and Allied Diseases, New York, N. Y (Introduced by J. H. Burchenal).

The oncolytic effect of viruses has been under laboratory and clinical study for several years. Because laboratory experiments indicated that Mengo, Bwanba and Semliki Forest viruses have broad and efficient oncolytic effect against human cancer cells in tissue culture or animal hosts, these viruses are receiving clinical trial in volunteer patients with advanced cancer. The virologic and serologic data from these studies are presented because human infection with these viruses has seldom been observed.

Semliki Forest virus was inoculated into 7 people. Viremia was demonstrated in 4. One had suggestive signs of encephalitis with complete recovery. Two had low grade fever without other clinical signs. There was no clinical indication of infection in the others. Virus was recovered from tumor tissue in 3 but there was no apparent effect on tumor growth. Virus was not detected in urine, feces, throat washings or cerebral spinal fluid. Pre-existing antibodies to West Nile virus did not prevent infection by Semliki Forest virus. Antibody formation was demonstrated by neutralization techniques. At autopsy no pathologic lesions attributable to the virus infection were detected.

Bwamba virus was inoculated into 3 people but was never recovered from blood, urine, feces, throat washings or cancer tissue. Fever occurred in 2 patients and acute laryngitis in one, but the relationship to Bwamba virus was not proved. There were no lesions attributable to virus at autopsy.

Mengo virus was inoculated once. Virus was recovered only from a blood specimen collected on day 2. There was no clinical reaction. Antibody formation was demonstrated.

These studies are continuing and results will be brought up to date at the meetings.

74. *Observations on the Epidemiology of Dengue in Panama.* LEON ROSEN, National Institutes of Health, Bethesda, Md.

In 1954 an epidemiologic investigation of dengue was undertaken in Panama, an area in which this disease was suspected to be endemic.

By means of both hemagglutination-inhibition and neutralization tests it was found that the extensive epidemic of a dengue-like disease which had occurred in 1941–1942 in Panama City and elsewhere was apparently due to dengue type-2 virus. These studies also indicated that this virus disappeared from these areas after the epidemic, in spite of the continued presence of a suitable vector, *Aedes aegypti*.

Studies on numerous sera from persons living in various forested areas of the country, and from wild-caught monkeys, yielded no data which suggested that either type of dengue virus was endemic in these areas.

75. *Philippine Hemorrhagic Fever; Etiological Relationship to Dengue.* W. McD. Hammon, Albert Rudnick, G. E. Sather, K. D. Rogers, L. C. LaMotte, Jr., Veronica Chan, and J. J. Dizon, University of Pittsburgh, University of the Philippines, and Department of Health, Republic of the Philippines.

During the past 5 years a severe and occasionally fatal hemorrhagic disease occurring almost exclusively in children has been recognized with increasing frequency on Luzon, particularly in and around Manila and other urban areas. Because of the dramatic clinical manifestations of fever, extensive petechial rash, epistaxis, gastrointestinal bleeding and shock this has been considered a distinctly new disease entity. It has a distinct seasonal pattern of highest prevalence in August and September. Lymphadenopathy, splenomegaly and leucopenia are absent or present to only a slight degree.

Dengue has never been diagnosed in a Filipino although outbreaks in foreigners have occurred in the past. Most recent and last outbreaks were during World War II among both Japanese and Americans. Dengue antibodies were found in a high proportion of Filipino children in 1953, giving impetus to this current study.

Clinical, epidemiological and laboratory studies were initiated in the summer of 1956. Approximately 750 cases were reported in the Manila area with about 15% case fatality. Cases appeared to have an association with the presence of *Aedes aegypti*. Many cases of febrile illness, with and without rash, petechiae or hemorrhage in children and young adults were found to have rising hemagglutination-inhibiting antibody titers to dengue, usually exceeding 1:2560. A number of viruses have been isolated by inoculation of suckling mice with acute-phase blood or with *Aedes aegypti*. Mice recovering from infection, during virus adaptation, resist intracerebral challenge with dengue viruses types 1 or 2. Experimental human convalescent dengue sera, types 1 and 2, neutralize the one virus tested to date. Isolations, identification and serology are still in progress.

76. *Trinidadian Virus Transmission Studies with Local Mosquitoes.* Thomas H. G. Aitken and Charles R. Anderson, Trinidad Regional Virus Laboratory, Port of Spain, Trinidad.

Arbor viruses isolated in the past in the island of Trinidad include the following known agents: yellow fever, dengue, Venezuelan equine encephalitis, St. Louis, Ilheus and Mayaro. Of these, only the latter 3 appear to be presently active in the human or animal population.

Studies have been initiated to determine the infectibility of local mosquitoes by these 3 viruses, through attempts to propagate virus and attempt transmission by bite. It has been demonstrated that certain species may harbor these agents for considerable periods of time. Transmissions were successfully effected by various species of *Culex*, *Aedes*, *Psorophora* and *Mansonia*.

*77. *Association of Encephalitis Virus with* Nippostrongylus muris *in Mice.* Geoffrey M. Jeffery, National Institutes of Health, Columbia, S. C.

78. *The Treatment of Enterobiasis in Children. A Comparative Study of Piperazine (Antepar) and Pyrrovinyquinium chloride (Vanquin).* Thomas S. Bumbalo, Lois J. Plummer and J. Roger Warner, University of Buffalo and Edward J. Meyer Memorial Hospital, Buffalo, N. Y.

The incidence of pinworm-infected children in a community remains high, notwithstanding the many studies reported on the efficacy of piperazine in treating pinworm infection. The authors are of the opinion that *simplification* of the treatment regimen would contribute to a decrease in the incidence of the infection.

The object of this study was to compare the efficacy of pyrrovinyquinium chloride, a comparatively new oxyuricide, with piperazine, a well proved drug, given in full doses in shorter courses of treatment.

Of the 195 children in the Immaculate Heart of Mary Home, Buffalo, New York, 139 (71%) were found to be infected with pinworms and were treated with either piperazine or pyrrovinyquinium chloride.

The percentage of cures in a group of 34 infected children treated with a single daily dose of 3 gm of piperazine for 3 consecutive days was 90.6%. The cure rate in a group of 34 infected children treated with a single dose of 3 gm of piperazine the first day and repeated on the sixth day was 86.2%. The cure rate of a group of 35 infected children treated with 1.5 mg/kg of pyrrovinyquinium chloride daily for 6 days was 100%. The cure rate of a group of 36 children treated with 2.1 mg/kg of this drug daily for 6 days was 97.1%.

Both drugs were well accepted and tolerated, and no untoward or toxic reactions were experienced by any of the children treated with these drugs.

79. *Chemotherapy of Strongyloidiasis with pyrrovinyquinium (Vanquin).* H. W. Brown and

* Publication withheld at request of author.

M. M. STERMAN, Columbia University and City Health Department, New York, N. Y.

A group of patients harboring *Strongyloides stercoralis* was treated with pyrrovinyquinium. Adults were given 50 mg 3 times a day for 7 days. Nausea was experienced by many of the patients, occasionally accompanied by vomiting and abdominal pain. Approximately 80% of the patients have remained negative for several months post-treatment. Gentian violet had been used unsuccessfully in a number of these patients.

80. *Relationship of Clinical Features to Immunologic Reactions in Visceral Larva Migrans.* R. C. JUNG AND G. PACHECO, Tulane University, New Orleans, La.

Patients with known larval *Toxocara* infection or a clinical diagnosis of visceral larva migrans were skin-tested with antigens consisting of aqueous extracts of *Ascaris* and *Toxocara* adult worms and larvae, and the results compared with similar tests in normal individuals and in patients supposed to be infected with *Ascaris* only. Tests with all of the antigens tended to be positive in patients with eosinophilia, and positives were frequently seen in normal controls. Positive skin reactions were less common in controls from northern states than from Louisiana. The skin-test did not appear to be of value in distinguishing *Ascaris* from *Toxocara* infections. Sera from patients with ascariasis or presenting eosinophilia and other findings suggestive of visceral larva migrans were tested for precipitins, using aqueous extracts of *Toxocara* and *Ascaris* as antigens in agar-diffusion and hemagglutination technics. In 7 cases in which the clinical impression was visceral larva migrans, antibodies were demonstrated to both *Ascaris* and *Toxocara* antigens. In 2 such cases antibodies to *Toxocara* only were demonstrated, and in one antibodies only to *Ascaris* were found. No *Toxocara* larvae could be demonstrated in tissues removed from the latter patient at autopsy, but he did have intestinal ascariasis.

*81. *Death of Dogs after Inoculation of Extracts from Cuticle of* Ascaris lumbricoides. JOSÉ OLIVER-GONZALEZ AND ANGEL CINTRON-RIVERA, University of Puerto Rico, San Juan, P. R.

82. Echinococcus *in Man and Dog in the Same Household.* M. M. STERMAN AND H. W. BROWN, City Health Department and Columbia University, New York, N. Y.

An *Echinococcus* cyst was removed from the liver of an American housewife of Greek extraction. In the course of epidemiological studies the adult forms of *Echinococcus* were found in a dog which the patient had imported from Greece 2 years previously. The infection in the dog was apparently unrelated to the infection in the woman. Casoni skin tests on 9 casual and intimate contacts with the infected dog revealed one positive reaction. The serums of all the contacts gave negative complement-fixation reactions, and x-ray studies and physical examination failed to show evidence of infection in any of the contacts.

83. *On the Human Helminthic Infections in Changua, Taiwan, China.* H. F. HSÜ AND S. Y. LI HSÜ, State University of Iowa, Iowa City.

Stool examinations of 2,012 persons in 7 villages in Changua, Taiwan, were made by the sedimentation method. The incidence of helminthic infections was recorded. In one village, egg counts for *Ascaris*, hookworm, and *Trichocephalus* were made in 613 cases. In addition, 20 cases of hookworm were treated with hexylresorcinol and the worms collected for specific determination.

The significance of these infections in Taiwan will be discussed.

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* Abstract withdrawn by authors.

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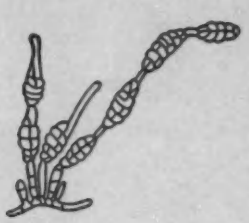
DIFCO

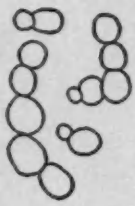
Media for

MYCOLOGY



DIAGNOSTIC

. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:



Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Maltose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

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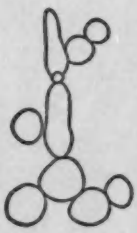

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Bacto-Yeast Carbon Base
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